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**THE NUTRITIVE VALUE OF DIFFERENT  
WHEAT VARIETIES FOR BROILER CHICKENS**

**ANNE  
LUCY A. WALDRON**

**A thesis submitted in partial fulfilment of the  
requirements of the Open University  
for the degree of Doctor of Philosophy**

**January 1997**

**Harper Adams Agricultural College**

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## ABSTRACT

The nutritive value of two UK wheat varieties, Dean and Beaver, from three different harvest years was assessed. A series of laboratory analysis and animal feeding experiments were conducted to examine the relationship between chemical composition, grain quality, and starch digestibility characteristics and the productive performance of broiler chickens fed wheat-based diets.

Differences were observed in the protein content, Hagberg falling number, hardness, particle size and specific gravity between the wheat varieties. Investigations into the nature of the carbohydrate in each sample revealed significant varietal differences in the amount of free glucose and the ratio of amylose to amylopectin in the endosperm. These results indicated that grains from the variety Dean had a harder endosperm texture, contained more starch packed into larger granules that were composed of proportionally less amylose, and had a lower  $\alpha$ -amylase activity than Beaver.

Feeding experiments using 7-21 day old broiler chickens confirmed varietal differences in broiler growth characteristics and feeding efficiency that were consistent over harvest years. These differences were not related to the apparent or true metabolisable energy values obtained for the experimental diets or the chemical composition of the wheat samples. A significant relationship between feed conversion ratio and Hagberg falling number and between weight gain and specific gravity of the wheat was attributed to improved starch solubilisation and the level of starch filling in the grain respectively, and suggested that the nature of the starch may have influenced the growth of the broiler. A relationship between the free glucose content of the wheat and broiler weight gain and feed intake was deemed to be a reflection of varietal differences in Hagberg falling number, and too small a difference to exert an influence on broiler performance.

Significant differences in broiler growth due to wheat variety were found in broilers aged from 7-49 days old, confirming that varietal differences in broiler performance occur throughout a typical commercial growing period. Investigations revealed no significant relationship between digesta viscosity and FCR. The digestibility of the wheat samples was examined *in vivo*, but was of little value as a measure of digestion differences due to high variation in results between individual animals. Large variety differences were observed, however, using an *in vitro* method that simulated the digestive processes of monogastric animals. These differences were strongly related to the weight gain, feed intake and FCR of the 7-21 day old broilers when the rate of digestion was less than 34 mg/min/100g, although after this point the relationship was random. Multiple regression analysis revealed that only the true metabolisable energy value significantly improved the linear relationship between the rate of starch digestion and broiler weight gain.

The relationship between rate of starch digestion and broiler growth was tested as a possible predictive method of wheat nutritional quality using six variety samples harvested in 1994, fed in pellet or meal form, to 7-21 day old broiler chickens. Significant performance differences between the varieties and diet forms were observed, but there were no significant relationships between performance and rate. The *in vitro* rate of starch digestion method could not therefore be verified as an accurate predictor of nutritive value of wheat for poultry.

## **DECLARATION**

This thesis was composed by the author and is a record of work carried out by her on an original line of research. All sources of information are shown in the texts and listed in the references; all help given by others is indicated in the acknowledgements

None of this work has been presented in any previous application for a degree.

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I would like to thank my supervisors Dr. Paul Rose and Mr. Peter Kettlewell, and Dr. S. Akeyasekera of Reading University Statistics Department for their help and advice throughout this project. I would also like to thank Chris Gee, Richard Page and Janet Pemberton, Dr. Jayne Powles, and Dr. Will Turner for their assistance with laboratory analysis, and Dave Carmichael, Justin Collier, Yuan Curtis, Jane Etheridge, Kate Harvey, Mac and especially John Protheroe for their help with the feeding experiments. I am grateful to Mr. S.P. Watkinson for supplying the grain from the 1990 harvest in Yorkshire, and I would like to acknowledge the assistance of Weston Research Laboratories for providing the particle size analysis and Finnfeeds International Ltd for the generous loan of the Brookfield Digital Viscometer. I would especially like to thank Nick Tucker, without whose tireless patience, support and proof-reading skills, this thesis would never have been finished.

This thesis is dedicated to my parents and to Nick.

## **PUBLISHED WORK**

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Waldron, L.A., Rose, S.P. & Kettlewell, P.S. (1994). Nutritive value of wheat for poultry with respect to wheat variety and growing year. *British Poultry Science*. **35**: pp. 829.

Waldron, L.A., Rose, S.P. & Kettlewell, P.S. (1994). The effects of feeding two wheat varieties from two growing years on the productive performance and digesta viscosity of broiler chickens at three ages. *Proceedings of the 9th European Poultry Conference*. Vol. 1: pp. 485-486. WPSA, Glasgow.

Waldron, L.A., Rose, S.P. & Kettlewell, P.S. (1995). Rate of *in vitro* starch digestion of two wheat varieties and its relationship to broiler chicken productive performance. *British Poultry Science*. **36**: pp. 875-876.

Waldron, L.A. & Rose, S.P. (1996). Rate of starch digestion and the nutritional value of wheat varieties. *Proceedings of the 20th World Poultry Congress*. Vol. IV: pp. 169-170. WPSA. New Delhi.

## LIST OF ABBREVIATIONS

AM	Amylose
AM:AP	Amylose to amylopectin ratio
AME	Apparent metabolisable energy
AMEn	Apparent metabolisable energy corrected for retained nitrogen.
AP	Amylopectin
DF	Degrees of freedom
DM	Dry matter
FCR	Feed conversion ratio
HFN	Hagberg falling number
NIR	Near infra-red reflectance
NSP	Non-starch polysaccharide
SEM	Standard error of the mean
TME	True metabolisable energy
TME <sub>n</sub>	True metabolisable energy corrected for retained nitrogen.



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ii-

# **1. INTRODUCTION**

## **1.1. GENERAL INTRODUCTION**

Milled wheat is the most commonly used cereal for broiler diets in the UK. Wheat typically contains between 60 and 70% starch and is fed to broiler chickens primarily as a low-cost source of readily digestible energy. It contains low levels of anti-nutritional compounds, compared to other cereals, enabling inclusion in broiler rations as a large proportion of the formulation, while still maintaining commercially acceptable levels of broiler growth and feeding efficiency (Graham, 1995). Protein content is high in wheat (Pettersen, 1988) in comparison to other commonly-fed cereals such as barley, but it has a poor amino acid profile with respect to broiler requirements, with inherent deficiencies in methionine and lysine. Wheat protein is of secondary consideration to starch content and availability since broiler diets can be supplemented with other cheaper and more digestible protein sources such as soyabean meal in order to meet the nutritional needs of fast growing broiler chickens. Typically, milled whole wheat provides 55% of the metabolisable energy and 35% of the protein requirement in complete diets formulated for broilers.

More than 450 million broiler chickens are produced in the UK alone each year (MAFF, 1995). As British poultry diets can contain between 40 and 70% wheat, the nutritional value and variations in quality of feed wheat are very important commercially.

Variation in the chemical composition (Graham, 1995) and broiler performance (March & Biely, 1973; Rose *et al.*, 1992) have been reported for different wheat samples. It has been suggested that the nutritional value of wheat is genotypically determined, and that broiler performance may be related to the variety used in the ration. Broiler performance variation may be a function of the structure and composition of the grain, or of interactions between these factors and the digestive chemistry and physiology of the bird. Previous investigations have yet to fully determine the relationship between measurable characteristics of wheat and the feeding efficiency of broiler chickens.

The general objective of this project was to examine the nutritional and chemical characteristics of the two wheat varieties, Dean and Beaver, that had previously been shown to give different weight gains when fed to growing broiler chickens (Rose *et al.*, 1993). Specific project objectives are listed at the end of the literature review (Chapter 1).

## **1.2. THE WHEAT GRAIN AS A FEED FOR POULTRY**

### **1.2.1. Cereal Grain Structure**

A grain of wheat can be divided into three basic units, the seed coat (bran layer), the germ or embryo and the endosperm, which contains the food reserves for the embryo. A mature, whole grain of wheat comprises 13% bran layer, 2% embryo and 85% endosperm.

Information that has no specific reference in the text has been derived from one of the following sources: Physiology & Biochemistry of Seeds (Bewley & Black, 1978); Biological Functions of Carbohydrates (Candy, 1980); Biochemistry of Seed Storage Carbohydrates in Green Plants (Dey & Dixon, 1985); Seed Biology (Kozlowski, 1972) or Seed Storage Compounds (Shewry & Stobart, 1993).

A generalised diagram of the structure of a whole wheat grain and a cross section through the grain layers are given in Figures 1.1 and 1.2 respectively.

### **(i) Seed coat**

The seed coat, or bran layer, of wheat is formed from structural carbohydrates and constitutes the majority of the crude fibre of the grain. It is composed of cellulose, lignin and non-starch polysaccharides (NSP), and is formed from the fusion of the pericarp and testa. The pericarp is a layer of cellulose impregnated with minerals, and forms the tough outer layer of the seed coat. The inner layer is formed from the testa, and gives the seed its colour. To maintain the viability of the ungerminated embryo through dormant periods the seed coat possesses a degree of water and gas permeability. It therefore able to perform a regulatory function, influencing both the development and metabolism of the seed.

### **(ii) Aleurone layer**

During seed maturation the peripheral cells of the endosperm multiply to form small rectangular cells in one or two layers. The walls of these cells then thicken and produce protein bodies. These layers of cells form the aleurone layer, and the protein bodies the aleurone grains. The aleurone layer contains less cellulose than either the pericarp or the testa, but the protein bodies within it are of considerable nutritive value to

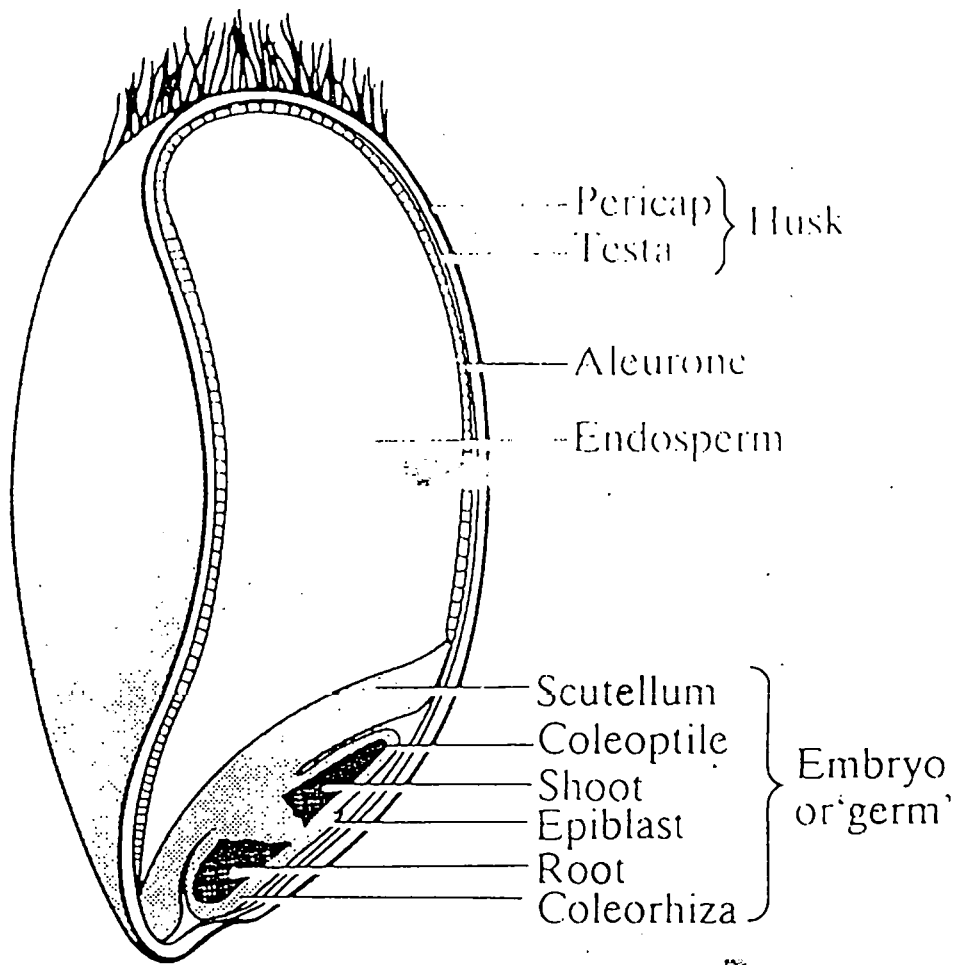


animals, though are often lost through removal of the bran (and with it the aleurone layer) by dehulling processes. The aleurone cells remain viable - unlike the inner endosperm cells which die, and then become packed with starch and protein - and thus play an important role in the breakdown of starch upon wheat germination. The aleurone layer is the storage area for the inactive  $\alpha$ -amylase enzyme which is responsible for the digestion of stored endosperm starch into glucose to supply energy to the germinating embryo (Stevens *et al.*, 1988).

The protein bodies ('aleurone grains') are well-defined structures within the aleurone layer, and associated with these aleurone grains is a chemical called phytin. A potassium, magnesium and calcium salt of myoinositol hexaphosphoric acid, phytin is found as a separate structure within the aleurone grains. It acts as a major store of phosphate and macronutrient mineral elements within the cereal grain.

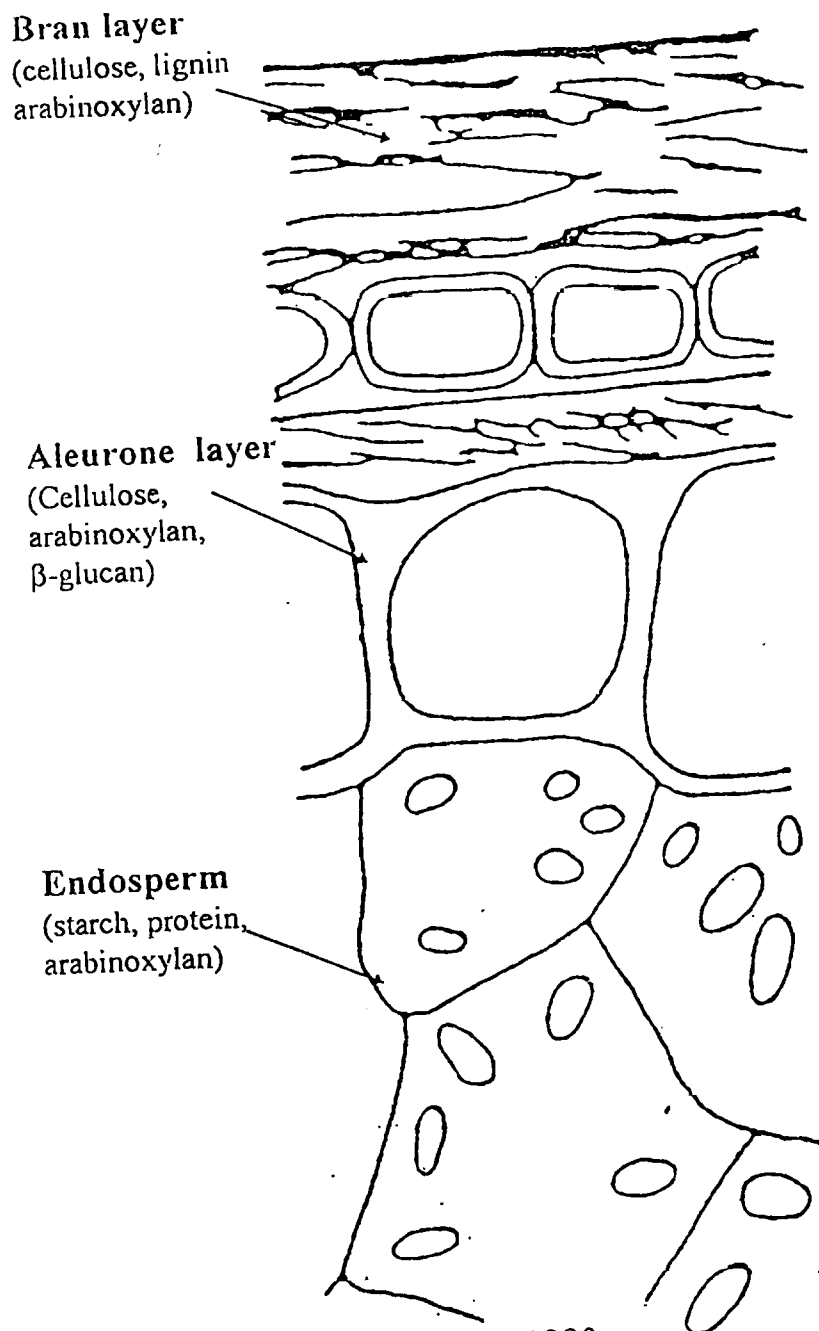
Oil bodies surround the aleurone grains and are also found as a narrow layer inside the plasmalemma; oil bodies form the triglyceride reserves in the grain (i.e. fat content). The size of these sub-cellular

FIGURE 1.1. WHEAT GRAIN STRUCTURE.



From McDonald, Edwards & Greenhalgh, 1981

FIGURE 1.2. CROSS SECTION THROUGH A WHEAT GRAIN.



From Selvedran & Robertson, 1990

organelles is dependent upon species and can vary between 0.2-0.6  $\mu\text{m}$  in diameter.

### **(iii) Endosperm**

The endosperm (i.e. that which does not form the aleurone layer at maturity) is the starch rich part of the grain that supplies the germinating embryo with protein and energy for growth. Wheat grains can contain up to 60-85% endosperm by weight, thereby forming the largest portion of the grain.

The honeycomb structure of the endosperm is formed from the cell walls of the dead parenchymous core cells, with the polysaccharides  $\beta$ -glucan and arabinoxylan contributing to the structural matrix. Organelles called amyloplasts, present in the cells of the developing seed, generate the starch granules that are then packed into the cavities of the endosperm.

The storage proteins in the endosperm are closely associated with the starch granules. The main storage protein is gluten, a substance which is made up of the smaller protein units (prolamins) gliadin and glutelin. As

the grain matures the accumulating starch granules disrupt and disperse the proteins, the protein forming a matrix around the starch granules in the mature grain.

The different types of protein that are present within the wheat grain can be assessed using amino acid analysis. There are differences in the amino acid content of the endosperm and the aleurone layer, with the endosperm containing less arginine than the aleurone layer.

#### **(iv) Embryo**

The majority of the protein within the cereal grain is contained in the embryo - the embryo comprises 1.5% of the grain weight - whilst the scutellum membrane contains 59% of the total thiamine content of the grain. The embryo also contains a limited amount of food reserves in the form of oligosaccharides, 20% of which consists, in a defatted wheat embryo, of sucrose (11.7%) and raffinose (8.3%) sugars.

### **1.2.2. Cereal Grain Composition**

The structures that constitute a wheat grain are composed from building blocks of different classes of molecules, these classes being, in broad terms, carbohydrate, protein and fat. A grain of wheat contains approximately 13% water, 10% protein, 2% fat, 68% carbohydrate and 5.7% cellulose.

#### **(i) Carbohydrate**

Carbohydrates perform both structural and storage functions. The majority of carbohydrate found in wheat is in the form of starch, the main function of which is energy storage. The other carbohydrates mainly perform structural roles in the endosperm matrix or testa and aleurone layers. Cellulose, although an important component of entire plants, is mainly present in the outer testa of the grain and has not been the subject of investigation in this study, and so the following section will concentrate on the major grain polysaccharides; starch and NSPs.

Starch comprises 64-74% of the endosperm in wheat grains. It forms a readily available energy source. The starch is contained in the wheat grains as granule structures and is formed from two polysaccharide molecules, amylose and amylopectin. A highly detailed review of the biochemistry of cereal starch has been published by Kainuma (1988), from which most of the following is derived.

#### **(a) Amylose and amylopectin**

The two carbohydrate molecules that make up wheat starch are both composed of glucose units. However they have different structural features. Amylose is a linear molecule comprised of glucose units joined together by  $\alpha$ -1-4 glucosidic linkages (figure 1.3). It has a molecular weight of  $14 \times 10^3$  g/mol. Amylopectin is also made up of glucose units, but is branched instead of linear. The main structure is formed by  $\alpha$ -1-4 linkages, but has  $\alpha$ -1-6 branched linkages every 20-25 units (see figure 1.4). It has an approximate molecular weight of  $4 \times 10^6$  g/mol and is therefore much heavier than amylose. The proportions of each of the two polymers in the starch

FIGURE 1.3. AMYLOSE

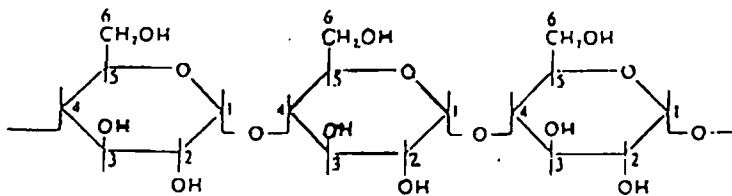
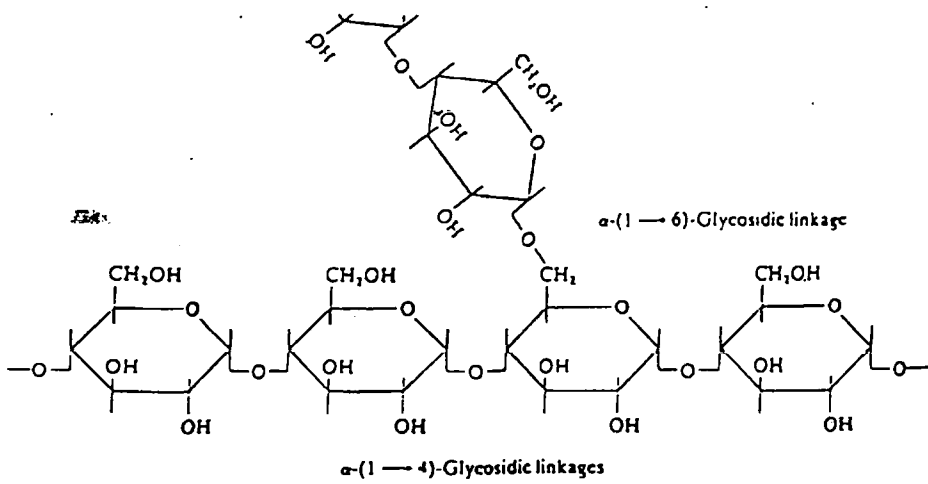


FIGURE 1.4. AMYLOPECTIN





varies between cultivars and growing years, and is related to the type and numbers of starch granules in the endosperm (Bewley and Black, 1978).

### **(b) Starch granules**

The number of starch granules in the endosperm is related to final grain weight (Chojecki *et al.*, 1986), making the number and size of the starch granules a determinant of the capacity of endosperm to accumulate starch, and thence the final calorific value of the grain.

A mature wheat grain contains starch granules that are usually classified into two size categories, A and B (large and small). More recently, however, this has been expanded to three categories, A, B and C (large, medium and small), for more clarity (Bechtel *et al.*, 1990). The growth and development of starch granules has been well documented over the last few years, and an in-depth review of the subject has recently been published by Morell *et al* (1995). Endospermic structures called amyloplasts synthesise starch granules. Type A granules are synthesised first, at about 4 days after the wheat plant flowers, and continue to grow until at least 19 days after flowering to a maximum size of between 25 and 50  $\mu\text{m}$  in diameter. Smaller type B granule growth is initiated at about 10

days after flowering and continues for a further 18 days until an average size of 9  $\mu\text{m}$  is reached at approximately 35 days after flowering. In the first 10 days of type B granule growth, development is slow due to the simultaneous accelerated growth (and corresponding increase in raw material demand) of type A granules. At 21 days after flowering type C granules begin to develop. These are the smallest of all the starch granules, and only develop to a maximum diameter of 5.3  $\mu\text{m}$ . In the mature grain, type A granules form, on average, 51.6%, type B 45% and type C 3.4% of the total mass of starch (Bechtel *et al.*, 1990).

In research comparing two wheat varieties, Chojecki and his co-workers (1986) found that certain chromosomes are responsible for grain weight and the types of starch granule present within the endosperm and therefore that this characteristic can be genetically selected. 'Soft' biscuit and feed type genotypes of wheat tend to have larger proportions of small B and C granules than the 'hard' milling varieties (Bechtel *et al.*, 1993).

The shape of starch granules is dependent upon the amount of amylose present. Granules with proportionally larger amounts of amylose compared to amylopectin in the starch result in a more rounded shape

whereas those containing more amylopectin than amylose are more flattened (Morell *et al.*, 1995). Most commonly, starch granules contain approximately equal quantities of both polysaccharides, which results in lens-shaped granules. Smaller granules tend to have higher levels of amylose and are therefore often rounder than type A granules (Bewley and Black, 1978).

### **(c) Influence of $\alpha$ -amylase activity**

Each cereal grain has its own enzymes that are responsible for the hydrolysis of endospermic starch, releasing energy for growth of the developing embryo when sprouting begins (Stevens *et al.*, 1988).  $\alpha$ -amylase in the aleurone layer performs this role in wheat (see section 1.2.1.) and has the same hydrolytic activity and cleavage sites as pancreatic enzymes found in animals, whereby it digests starch molecules by splitting the  $\alpha$ -1, 4 glycosidic bonds to form short chain oligosaccharides and limit dextrins. Plant  $\beta$ -amylase then further reduces these digestion products to their glucose subunits (Gruppen, 1996). When germination begins, due, for example, to cultivation or exposure to moisture in poor storage conditions, the resulting  $\alpha$ -amylase activity and

subsequent starch digestion will reduce the quantity of available starch within the grain and the embryo will develop.

Milling wheats with hard endosperm textures have lower  $\alpha$ -amylase activity than soft wheats, making this a genetically determined property of cereals. The  $\alpha$ -amylase content of wheat is widely used as a quality measurement, and is determined by the Hagberg falling number method. The Hagberg falling number (HFN) is calculated using a method first devised in the early 1960s by Sven Hagberg (Hagberg, 1960, 1961). Wheat flour is mixed with water and gelatinised by heating. A plunger is then allowed to drop, under the force of gravity, through the mixture; the time (in seconds) taken by the plunger to pass through the mixture is recorded. This is the Hagberg 'falling number'.

High  $\alpha$ -amylase activity within the mixture causes liquefaction of the starch, and thus lowers the Hagberg falling number (Pertin, 1964; Best & Muller, 1991). High quality bread wheats with hard endosperm and low  $\alpha$ -amylase activity have high HFN's and soft wheats with high  $\alpha$ -amylase activity have low HFN's.

#### **(d) Non-starch polysaccharides**

The other important group of carbohydrates present in wheat are the NSPs, which perform a structural role and account for approximately 10% of the whole grain. The majority of these compounds are found in the bran layer (Theander *et al.*, 1993). This group of polysaccharides is mostly composed of the mixed-linked  $\beta$ -glucans and arabinoxylans, which are apportioned to different areas of the grain due to their functional roles. The proportions of arabinoxylans and  $\beta$ -glucans present within grain is dependent upon the species of grain. For example, barley and rye contain both, whereas oats mainly contain  $\beta$ -glucan. In comparison with these cereals wheat contains relatively small amounts of NSP due to its low  $\beta$ -glucan content, as can be seen in the comparison between the barley and wheat NSP content is shown in table 1.1 below.

Most NSP in the whole wheat grain is in the form of insoluble arabinoxylan (55 g/kg) (Englyst *et al.*, 1989).

**TABLE 1.1. COMPARISON OF THE NON-STARCH POLYSACCHARIDE CONTENT OF WHEAT AND BARLEY**

Non-starch polysaccharide (g/kg)	Wheat	Barley
Soluble $\beta$ -glucan	5.2	33
Insoluble $\beta$ -glucan	6	9
Soluble arabinoxylan	15	13
Insoluble arabinoxylan	54.5	52.1
Other soluble NSP	3	3
Other insoluble NSP	5	5
Total soluble NSP	23.2	49
Total insoluble NSP	65.5	66.1
Total NSP	88.7	115.1

From Cowan, 1996.

The total arabinoxylan content (soluble and insoluble) of cereal bran is generally between 200-400 g/kg of dry matter. They perform a major structural role and a minor carbohydrate storage role in wheat grains. The majority of arabinoxylans are present in the outermost part of the seed coat and are bound to lignin to give structural strength. They are found in smaller amounts in the endosperm and aleurone layers, where they form the main structural cell wall polymer.

Arabinoxylans are not homologous molecules and may show different levels of molecular branching and substitution. In a comparison between wheat and rye Theander *et al.* (1993) found that wheat flour had 25% of the soluble arabinoxylans content of rye, with a greater proportion being unsubstituted xylose subunits, but the same amount of di-substituted xylose units. These differences affect the physical and functional properties of the polymers, and will determine where they are employed in the grain and how they interact with other compounds.

$\beta$ -glucans are structural and storage polysaccharides that are similar to cellulose, containing 1-3 and 1-4 linkages. They are formed from two units that differ in the number of 1-4 linkages between each 1-3 linkage (Theander *et al.*, 1993).  $\beta$ -glucans act as a minor energy reserve as well as forming up to 10% (compared to 85% arabinoxylans) of the polysaccharide component of the endosperm, where they are layered onto the cell walls. This prevents cracking during dehydration and dormancy of the mature grain, and therefore contributes to drought defences in the seed. This is in contrast to the aleurone layer which contains up to 98%  $\beta$ -glucans where these carbohydrates are concentrated upon the inner layer, and they also form a large component of the bran layer of wheat (Dixon, 1985).

## **(ii) Starch / protein matrix**

The starch/protein matrix of the endosperm imparts the 'hardness' characteristic to cereals. Hardness is a measurement that is used as a guide for milling quality of each batch of wheat, whereby hard wheats tend to mill better than soft wheats. Hard wheats shatter when milled, giving fine flours with regular particle sizes and large surface areas which are desirable to the



baking industry. Soft wheats tend to get squashed flat in the mill, which gives poor, irregular flours with low available surface areas (Vincent *et al.*, 1995; Albertini, 1995).

Recent research into the endosperm anatomy of wheat showed differences between the starch/protein matrices of hard and soft wheats (Brennan *et al.*, 1993). The actual relationship and interaction between starch and protein is, as yet, unclear and currently there are conflicting reports on the relationship between hardness and starch granule size and number (Pitts *et al.*, 1989; Glenn *et al.*, 1992), although there are some properties which may be useful in predicting grain quality. Scanning electron microscopy has revealed that there is an association between the starch/protein matrix of the endosperm and endosperm texture. A protein called friabilin has been found to be associated with both internal and external structures of starch granules and thought to be involved in starch formation (Sulaiman *et al.*, 1993). This protein could be used to identify whether or not a wheat sample has a regular and even endosperm texture. Hard wheats show a highly regular, strong matrix between starch and protein, but the opposite is found in soft wheats (Sulaiman *et al.*, 1993). This would further support the theory that the

regularity of the matrix may be responsible for the 'shattering' of hard wheats during milling, as the protein part of the matrix would provide a plane for fractures to develop along (Vincent *et al.*, 1995). A certain fraction of friabilin known as "grain softness protein" associates more strongly with soft wheat starch granules (Jolley *et al.*, 1993), and could possibly be used as an indicator of relative hardness. Interactions between the granules and the surrounding protein may have some influence over the size and shape of the mature granule (Bechtel *et al.*, 1993), which could determine granule ratios (A:B:C).

### 1.2.3. Variation In Wheat Grain Composition

The composition of modern wheat cultivars can vary quite widely. For example, the amount of starch can fluctuate between 600 and 730 g/kg dry matter (Graham, 1995). Similarly the crude protein content range, on a dry matter basis, has been reported as varying between 80 and 140 g/kg (McDonald *et al.*, 1981) and between 97 and 170 g/kg (Graham, 1995). One of the earliest references to wheat variability was in 1949, when Schollenberger and Curtis reported differences in the chemical composition of a number of Canadian wheat samples. They found large differences in 1000 grain weight and crude protein, the latter also linked to variation in starch and sugar content. There were small differences between the wheat varieties; larger differences were observed between harvest years and growing sites. An example of the reported variability of the constituents of wheat of European wheat samples is given in table 1.2.

**TABLE 1.2. COMPOSITION (g/kg DRY MATTER) OF 15 SWEDISH WHEATS COLLECTED OVER 4**

**YEARS**

(Graham *et al.*, 1988)

Component	Mean (g/kg DM)	Range (min-max)
Starch	675	600 - 730
Crude protein	128	96 - 170
Total fibre*	126	89 - 180
Free sugars	31	16 - 54
Crude fat	24	19 - 30
Ash	16	11 - 19

\* Total fibre defined as arabinoxylan, cellulose,  $\beta$ -glucan and lignin.

## **(i) Variety**

The proportions of starch and gluten vary between wheat cultivars, gluten content being important in bread quality wheats, and has been heavily genetically selected in plant breeding. The structure and content of the starch grains are very important to the energy value and availability of the wheat. The protein contained in the embryo also varies between cultivars. Genetic manipulation of wheat can increase its protein content, which may prove useful as a source of protein in animal feed in the future (Wiseman *et al.*, 1993).  $\beta$ -glucan content has also been described as variety-dependant (Stone & Clarke, 1992), with some cultivars containing more than others. This may be due to differences in genetic selection for drought resistance amongst certain varieties of wheat

## **(ii) Environmental effects**

The structure and development of the wheat plant and its grain is obviously greatly influenced by the climatic conditions under which it is growing. There are environmental influences on the deposition and amounts

of starch laid down in wheat grains, whereby the synthesis of amylose has been shown to be reduced by high temperatures and drought conditions (Jenner *et al.*, 1991). Drought limits photosynthetic activity and production of carbohydrate subunits, which restricts the production of raw materials available for manufacture of starch within the endosperm. Soluble carbohydrates may be mobilised from the stem under such conditions to meet the shortfall, but in prolonged drought conditions the result is a decrease in the number of B granules, and the size of A granules, in the endosperm. Drought, and temperature above 30°C after flowering, may cause early cessation of starch deposition. However, drought immediately before harvest often results in an increase in starch content due to increases in deposition during the 'filling out' of the grain at the end of its development (Wiseman, 1990).

NSP level in cereals varies between harvest years (Saastamoinen *et al.*, 1989). Cold years have resulted in increased precipitation of arabinoxylans and hence an increase in the percentage of bran per grain (due to the smaller grain produced). Saastamoinen *et al.*, (1989) determined that this increase was linked to a decrease in HFN in rye grain, though this effect has yet to be

verified in wheat. The opposite effect is found in the case of  $\beta$ -glucans. Large grains contain more  $\beta$ -glucan, and do not appear to be affected by climate. Data indicating differences in  $\beta$ -glucan content between harvest years and growing sites was reported by Stone and Clarke (1992), who found that although temperature did not have an effect on the deposition of  $\beta$ -glucan, soil water content did. Dry conditions and low soil water levels during ripening were reported to increase  $\beta$ -glucan levels. Rainfall reduced  $\beta$ -glucan levels and correspondingly reduced flour viscosity (a characteristic of high  $\beta$ -glucan levels).

In summary, cereal arabinoxylan content appears to be more affected by environmental factors than  $\beta$ -glucans. In poor growing years the arabinoxylan content increases and the Hagberg falling number decreases, due to increased  $\alpha$ -amylase activity.  $\beta$ -glucan content shows a positive correlation with yield due to environmental factors.

### **(iii) Agronomic influences**

The husbandry and management of cereals during growth and harvesting will influence the quality and composition of the grain. Wheat sown late in the season has been found to have higher  $\beta$ -glucan levels, which may be due to ripeness. Such differences between wheat samples harvested at different times was reported for experiments conducted by Stone & Clarke in 1992. They reported that wheats harvested when still “yellow” had a higher  $\beta$ -glucan content than those harvested when fully ripe. The viscous properties of wheat flour appears to change with respect to maturation, a change probably linked to the final grain filling and starch deposition.

The timing of fertiliser application is also thought to affect the milling character of wheat grain. Nitrogen application and availability alters the deposition of the endosperm starch-protein matrix, affecting the hardness and texture of the grain (HGCA, 1993). Too little nitrogen, or nitrogen applied too late on a crop, will result in poor matrix structure and poorer endosperm strength.



### **1.3. THE DIGESTIVE PHYSIOLOGY OF THE CHICKEN**

Descriptions of feeding control and digestive processes are available in a great many textbooks, and where there is no reference in the following text, the information source was either Farmer (1960), McLelland (1979), Hill (1983), Sykes (1983), McDonald, Edwards and Greenhalgh (1988) or Larbier and Leclercq (1994).

The different components (protein, fat, starch) that make up the diets fed to commercial poultry are digested in different areas of the gut by specific enzymes and under specific conditions. The process of digestion is initiated with the ingestion of the feed through the mouth, and ceases with the excretion of undigested material through the cloaca.

#### **1.3.1 Mouth**

The process of digestion starts as soon as the animal begins eating, with the feed taken into the mouth being immediately covered in saliva from the salivary glands. Saliva contains mucus,  $\alpha$ -amylase and bicarbonate; the

mucus softens and moistens the feed whilst the bicarbonate ions buffer this mixture at a neutral pH. This facilitates the activity of the salivary  $\alpha$ -amylase, which begins the hydrolysis of starch into glucose subunits. The food is then transferred by peristaltic movement to the crop.

### 1.3.2. Crop

The crop acts as a reservoir, regulating transit through release of controlled amounts of digesta into the proventriculus for digestion. It is distension of the full crop that triggers neural pathways, signalling satiety to the bird and thereby regulating feed intake. The crop contains a microbial population made up of Lactobacilli species which produce exogenous  $\alpha$ -amylase to break down starch in conjunction with the salivary  $\alpha$ -amylase (Champ *et al.*, 1983). However, only starch that has been made available by feed processing will be digested in this part of the gut.

The  $\alpha$ -amylase acts by hydrolysing the  $\alpha$ -1,4-glucan bonds present in polysaccharides, but only where they contain more than three D-glucose units

linked together by such bonds. Glucose polymers of this type are to be found in starch, glycogen, and oligosaccharides.

### **1.3.3. Proventriculus**

The food passes from the crop to the proventriculus (which is sometimes referred to as the “chemical” stomach) where protein digestion begins. The protease precursor pepsinogen, along with hydrochloric acid, is secreted from glands in the proventriculus wall in response to stimulation resulting from the distension of the feed-filled crop. The addition of hydrochloric acid to the digesta lowers the pH of the proventriculus to between 1 and 2, activating the inert pepsinogen to pepsin, and ensuring optimum conditions for the proteolytic activity of the pepsin. Pepsin cleaves the carboxyl bonds of the protein to produce smaller polypeptides (Gruppen, 1996). The food only remains in the proventriculus for a short while before it passes to the gizzard.

#### 1.3.4. Gizzard

The gizzard is sometimes referred to as the “mechanical” stomach and has the primary function of grinding the digesta to a smooth paste. The walls of the gizzard are lined with a thick, horny layer of a proteinaceous material called koilin, which provides a grinding surface as the stomach contracts. This action may be increased by the addition of grit to the diet, being retained in the gizzard to aid the grinding of hard feed materials such as whole grains. Grinding reduces the particle size of large pieces of the diet, such as cereal hulls and large pieces of endosperm, and increases the surface area of the digesta components. The high pressure, shear forces and churning action, combined with the HCl from the proventriculus, act together to destroy tertiary protein structure, enabling the pepsin to hydrolyse the long chain proteins into simple subunits and constituent amino acids. Where the bird has been fed cereals, this action will rupture the starch granules, releasing amylose and amylopectin, which in turn will begin to solubilise by the cleavage of internal hydrogen bonds, and chelation with water molecules (Rogel *et al.*, 1987). This renders the cereal starch more vulnerable to attack

by carbohydrate enzymes in the duodenum and upper ileum. The digesta passes from the gizzard to the duodenum.

#### **1.3.5. Duodenum**

The duodenum and upper part of the ileum (jejunum) are the areas of the gut where carbohydrate hydrolysis predominates and nutrient absorption occurs. Mucus, electrolytes and enzymes are secreted onto the digesta in the duodenum from the lining of the tract, the pancreas and the gall bladder.

The duodenal lining secretes the enzyme  $\alpha$ -glucosidase. This enzyme hydrolyses simple sugars to their constituent glucose units as they pass through the length of the small intestine.

Bile secretions from the gall bladder raise the digesta to pH 6 and act as a surfactant to emulsify fats and lipids, enabling fat digestion to take place.

Pancreatic secretions contain enzymes, including the proteolytic endo- and exo-peptidases, trypsin and chymotrypsin (which complete the final

stages of protein digestion in the ileum), and starch hydrolytic enzymes ( $\alpha$ -amylase). Pancreatic lipase breaks down the lipid to fatty acids, which form micelles that are then solubilised and moved across the ileal brush border into the portal vein. These secretions also contain bicarbonate buffers that stabilise the pH at 6, promoting enzyme activity and quenching the activity of stomach enzymes.

### **1.3.6. Ileum**

In the ileum the pancreatic peptidases digest the polypeptides produced by pepsin activity into amino acids and peptides that are then absorbed through the gut wall (Gruppen, 1996). Pancreatic  $\alpha$ -amylase cleaves the  $\alpha$ -1-4 linkages of amylose and amylopectin to generate short-chain oligosaccharides. These are then broken down to glucose by oligo- and disaccharidases secreted from the ileal brush border membrane (Alpine & Sheshukova, 1992), and absorbed from the lumen (Gruppen, 1996). Maltose is hydrolysed to glucose by  $\alpha$ -glucosidase secreted from the duodenal lining.

The digesta moves down the length of the ileum whilst the processes of digestion and absorption continue, and after having passed through the small intestine (duodenum plus ileum) the main dietary components, i.e. starch, protein and fat, will have been digested. If digestion has been efficient, the digesta will contain only the 'fibre' part of the diet, and will do so since chickens do not possess the enzymes necessary to digest these molecules. At the end of the ileum, the digesta that remains enters the caeca.

#### **1.3.7. Caeca**

In chickens the colon is virtually absent and is replaced by two enlarged caeca that lead directly into the rectum. These contain large microbial populations which ferment any nutrients remaining in the digesta. The terminal zone of each caecum is the site of considerable bacterial activity.

Chicken hind-gut microflora are heterogeneously cultured in the caeca of the digestive tract. Here they are sheltered from the more disruptive activity of the main alimentary canal, enabling them to establish colonies and

digest food material more efficiently (Leitgeb, 1990). The dominant species are lactic acid bacteria and Streptococci (Kovalenko *et al.*, 1989) that require acidic conditions in addition to a supply of starch as a carbon source (Champ *et al.*, 1983).

Passage of food into the caeca is regulated by the muscles at the end of the ileum, that control the flow of digesta by peristalsis and muscle contraction. The microflora in the caeca ferment the undigested material (mostly fibre), producing volatile fatty acids which are then absorbed through the caecal lining (Chesson, 1990).

#### **1.3.8. Rectum**

After the digesta has been subjected to hind gut fermentation, water is reabsorbed from the remains of the feed. The digesta is now faecal material and passes into the rectum prior to being excreted.

The typical digestive transit time for a broiler chicken is four hours. However, this time is dependent on the age of the animal, its feed intake and early nutritional experiences (Tur *et al.*, 1986).



#### **1.4. FACTORS AFFECTING DIGESTION AND BROILER PERFORMANCE**

The dietary nutrients available for metabolism is a function of the ability of the animal to consume and digest feed efficiently. The chemical and physical properties of the dietary components (carbohydrate, protein and fats) have a direct bearing on the digestibility of the feed. For example, the more complex molecular structure of some nutrients (amylopectins, starch-lipid complexes) may result in poorer digestibility due to slower digestion rates than those with very simple structures, a factor which is independent of the energy content of that feedstuff (Pettersen, 1988). The variable nature of wheat grain composition (described in section 1.2.3 above) would suggest that the nutritional value for broiler chickens will vary between wheat samples. The following section discusses the influential roles of certain factors found in cereals that have been shown to, or may have a bearing upon the digestibility of feed and broiler productive performance.

#### 1.4.1. Starch and Energy Availability

The amount of glucose available to the bird as a ready energy source is limited by the quantity liberated from starch by the enzyme activity of the carbohydrases in the ileum (Levin, 1976). Glucose is required by the bird for maintenance of its metabolic and homeostatic mechanisms, and to assimilate and convert other nutrients into tissue. The ability of the broiler to digest the carbohydrate-rich cereal component of its diet therefore influences its capacity for growth (Mollah *et al.*, 1983). Certain proteins contained within the embryo of wheat grain have been found to inhibit the  $\alpha$ -amylase enzymes secreted by chickens for the digestion of cereal starch (Snow & O'Dea, 1981; Rogel *et al.*, 1987). The level of the inhibitor present in the embryo is determined by the genotype of the wheat (Warchalewski *et al.*, 1989). These proteins are usually destroyed by heat generated from friction during milling, and by the enzyme pepsin secreted in the gizzard (Snow & O'Dea, 1981; Rogel *et al.*, 1987; McNab, 1993), and therefore are thought not to interfere with starch digestibility in broiler chickens fed milled or processed diets (McNab, 1993).

Wheats that accumulate more starch and have heavier grain weights may well prove to have higher nutritive value for poultry, since provided that the digestibility of the starch is high, there will be more glucose potentially available (Mollah *et al.*, 1983). Graham (1995) identified those wheats containing large amounts of starch and low levels of fibre as being those best suited to the nutritional requirements of growing broiler chickens.

The differences between cultivars in grain filling and starch development within the endosperm could also dictate the availability of the starch for digestion within animals. It may therefore be useful to select certain wheat cultivars that show these quality characteristics (Chojecki *et al.*, 1986) for the production of higher nutritive quality broiler feeds, and consequently more efficient productive performance. Cultivars with more regular endosperm organisation and a greater number of 'A' type granules could also be of increased nutritive value to chickens (Wiseman, 1990) since this type is more readily gelatinised in the gut. Hard, regular endosperm structures tend to result in uniform availability of these large granules for enzymic attack. Larger, A-type granules are more easily ruptured during feed processing and digestion because of their surface to volume ratio, and

therefore the starch contained in those granules will be more readily available for hydrolysis by enzymes. The smaller B and C-type granules are more difficult to rupture (Gruppen, 1996) and often escape digestion due to their size and small surface area to volume ratio.

#### **1.4.2. Amylose and amylopectin**

The digestibility of cereal starch is dependent upon its amylose and amylopectin content (Rogel *et al.*, 1987; Gruppen, 1996). Amylose is considered to be more digestible than amylopectin because of its simple linear molecular structure. Although amylose is theoretically a readily available source of glucose, it has been shown to interfere with digestion in the ileum of monogastric animals (Behall *et al.*, 1989). Free amylose has the capacity to form chemical complexes with lipids and proteins, rendering them resistant to enzymic attack (Åman & Graham, 1990). This effect slows the rate of digestion and reduces the release of useable nutrients from the feed. The branched structure of amylopectin makes it poorly digestible by  $\alpha$ -amylase, which results in larger digestion products which require further hydrolysis before they can be absorbed from the lumen. These branching bonds prevent

complete digestion by  $\alpha$ -amylase, so only larger branched and unbranched oligosaccharides, which are rich in  $\alpha$ -1,6-glucosidic bonds, are formed as digestion products (Gruppen, 1996).

Differences in the digestion of the two starch polymers suggest that wheats containing more amylose in relation to amylopectin would be more readily digestible. However, the problems associated with the ability of amylose to form indigestible complexes with other nutrients, and thus slowing down digestion, would need to be considered (Behall *et al.*, 1989). There may be a relationship between the amylose:amylopectin ratio in wheat and the resulting rate of starch digestion and broiler growth, and from which it could be possible to predict the feeding value of wheat samples.

#### **1.4.3. $\alpha$ -amylase activity**

Diets formulated with wheat that has sprouted, due to increased  $\alpha$ -amylase activity caused by late harvesting or poor storage (Wiseman & Inborr, 1990), will have less nutritionally-available starch within the

endosperm; some of the starch will have been digested and the resulting glucose used as an energy source for tissue growth by the developing embryo. The amount of  $\alpha$ -amylase contained in wheat grains has been related to feeding quality for broiler chickens, in that wheat with lower enzyme activity gives better liveweight gains than those samples with higher levels of  $\alpha$ -amylase (Rose *et al.*, 1992; McNab, 1991). Rose *et al.*, (1992) found that there was a correlation between wheat HFN and the energy available to the bird (AME) from wheat diets, whereby wheats with higher HFN produced diets which were higher in AME. The reason for this was unclear, as the starch content and chemical composition of the wheat was not studied. More importantly (from a commercial viewpoint) they reported a trend between HFN and broiler growth whereby higher HFN wheats produced better liveweight gains. HFN may be related to the amylose: amylopectin ratio of the starch granules or the NSP content of the wheat, as both these characteristics would influence the solubility and viscosity of the wheat sample (Lund, 1984). The nature of the starch within different wheat samples would need to be investigated if the true relationship between these components is to be determined.

#### 1.4.4. Hind gut microflora

The micro-organisms of the caeca contribute to the enzyme activity of the gut, complementing the natural enzyme secretions of the bird, thereby increasing the types of dietary components that can be digested. For example, gut microflora will ferment both NSP and cellulose, in addition to any material that has not previously been digested (Champ *et al.*, 1983; Longstaff *et al.*, 1988; Schutte *et al.*, 1992; Annison, 1993). This supplementary enzyme activity was observed by Ishibashi (1983) who found that germ-free birds kept in sterile environments (to prevent development of microbial colonies within their gut) had lower gut enzyme activities than conventionally-reared controls. The host and bacteria coexist in a state of mutual symbiosis under ordinary circumstances, although the host is competing with the bacteria for the released nutrients (Annison, 1993).

Hind gut development is influenced by diet, and the types of colonising micro-organisms. Loss of, or reduction in microflora populations will reduce hind gut weight, as demonstrated by Henry (1987) when he fed antibiotics to chickens and measured caecal development.

Experiments by Meluzzi *et al.* (1986) showed that the activity of gut micro-organisms exerted significant influence over broiler productive performance. The fermentation of fibre by gut micro-organisms contributed to the amount of energy available to the bird for growth as a result of the production of metabolisable volatile fatty acids.

The effects of additional supplementation of broilers with common hind gut micro-organisms has been studied. When diets were supplemented with Streptococci or lactic acid bacteria the broilers had higher weight gains and better feed conversion ratios (FCR) (Meluzzi *et al.*, 1986). This may be explained by the later work of Muramatsu *et al.* (1987, 1993), who found that protein anabolism within the body of the bird increased in the presence of gut micro-organisms. The natural gut flora may therefore further contribute to broiler growth by assisting protein uptake and availability.



#### 1.4.5. Dietary fibre

Fibre components are defined as cellulose and lignin (from the bran layer) and the soluble and insoluble cell wall NSP, especially  $\beta$ -glucan and arabinoxylan, (Åman & Graham, 1990; Englyst *et al.*, 1992). Soluble NSP has been identified as an anti-nutritive factor in chicken diets that causes poor growth and feed conversion (Antoniou & Marquardt, 1981; Longstaff *et al.*, 1988; Bedford, 1992; Choct & Annison, 1990, 1992; Schutte *et al.*, 1992; Graham, 1994, 1995). Insoluble NSP is the portion of the diet that is commonly referred to as 'dietary fibre'. It has been reported that if pure dietary fibre, in the form of isolated soluble and insoluble NSP, is fed to broiler chickens there is an associated loss in growth and performance that is dose-dependant (Wiseman & Inborr, 1990; Choct & Annison, 1992; Annison, 1993).

Wheat does not usually contain levels of NSP as high as barley (Cowan, 1996) (see table 1.1), though variation between wheat samples is considerable. The variability of wheat, and the environmental effects on arabinoxylan content in wheat grains, may indicate that the nutritive value of

wheat could vary between harvest years as a result of changes in NSP level and profile. This would be reflected in differences in broiler performance not only between varieties but also between harvest years.

Wheat bran will have more, and dehulled cereals will have proportionally less, NSP when added into poultry diets. Formulations need to take account of the possible negative effects on digestion of adding certain wheat components into diets.

### **(i) Effects of dietary fibre on ileal digestibility**

Dietary fibre causes increases in the viscosity of the digesta in the upper ileum, where the NSP fibres solubilise and form a viscous gel. This gel can be visualised as a fine mesh of interwoven fibres that trap nutrients and water (Bedford, 1992; Annison, 1993; Cowan, 1996). The formation of the gel inhibits nutrient digestion as the endogenous enzymes of the broiler will have limited contact with the substrates that are caught in and surrounded by the network of fibres. The gel also has a large water holding capacity, and removal of water from the gut environment further reduces substrate contact and thence decreases digestion rate. The transit time of the digesta is increased and absorption across the gut wall reduced as the whole food mass slows down and becomes thick and sticky. As a consequence of the poor starch digestion and reduced glucose release from the feed, blood glucose levels will also decrease (Snow & O'Dea, 1981; Behall *et al.*, 1989).

The interference of the increase in viscosity with nutrient uptake and assimilation was quantified by Bedford and Classen (1992). They found that the digesta viscosity of wheat and rye-based diets was related to broiler

performance by a positive logarithmic relationship between viscosity and FCR. In diets formulated with barley or rye, which have a large soluble  $\beta$ -glucan and arabinoxylan content (Englyst *et al.*, 1989), viscosity is very high.

Broilers fed diets that result in high digesta viscosity, and hence slower movement of material within the digestive tract, can affect the distribution of caecal microflora. The slowed passage of digesta can promote the colonisation of the ileum by the bacteria (Rogel *et al.*, 1987).

The digestion and absorption processes in the ileum under conditions of microbial colonisation will be affected, the host being in direct competition for nutrients with the now much larger microbial population. This increase in competition could cause a decrease in nutrient availability to the host bird, and thence depress growth, as the bacteria would then be able to digest a greater proportion of the hydrolysed nutrients which were available to the bird (Rose & Bedford, 1995).

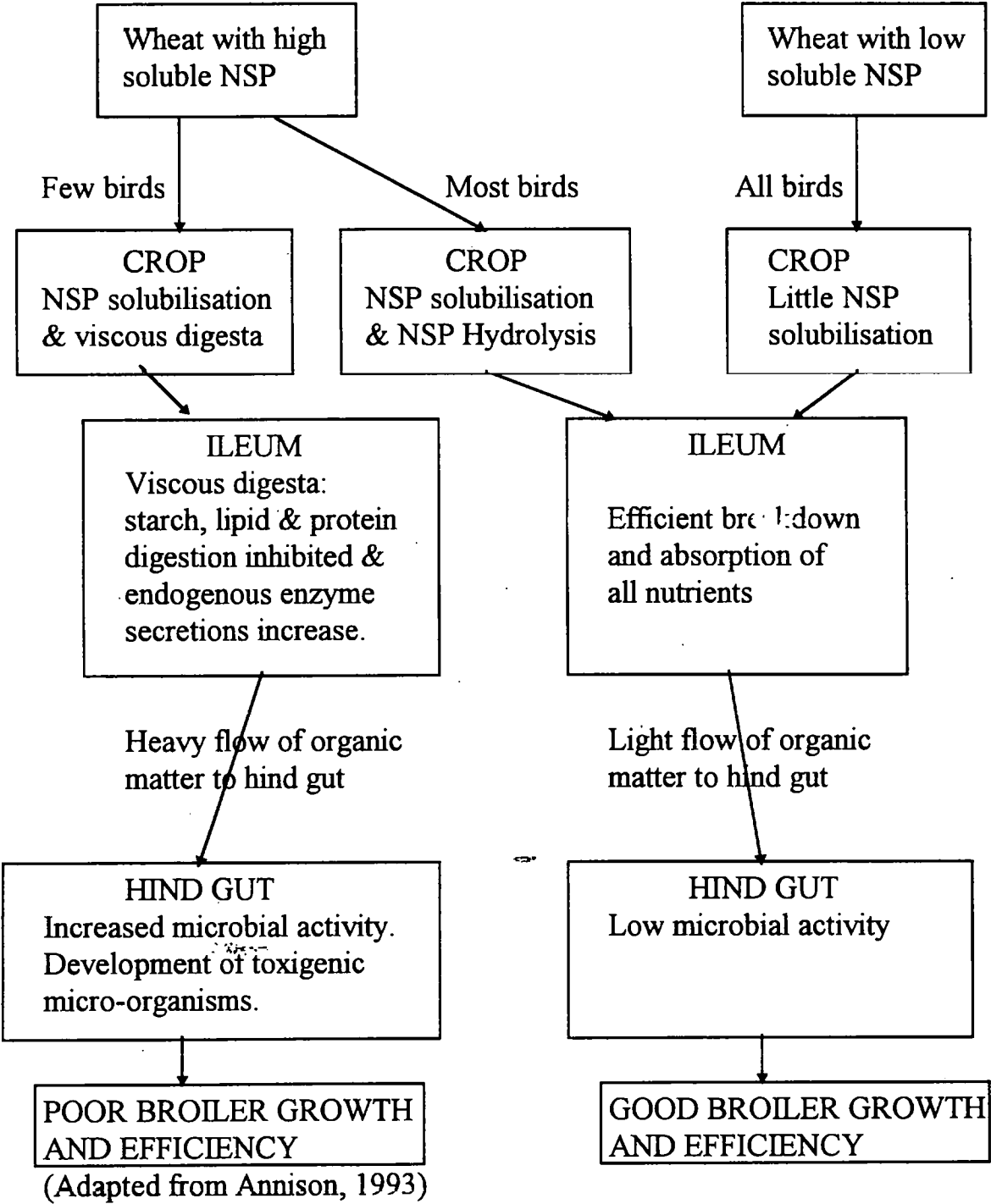
The presence of NSP within the diet can therefore alter digestion and absorption in the ileum, and limit the flow of nutrients to the animal.

## **(ii) Effects of dietary fibre on hind gut fermentation**

Birds fed on diets with high fibre contents have significantly higher caeca volumes (Kamar *et al.*, 1987a, 1987b), as a result of which more feed can be fermented each time the caeca are filled. It is therefore important that the diet has sufficient fibre to ensure healthy hind-gut development and colonisation with beneficial organisms, so as to complement endogenous enzyme secretion. The flow of undigested organic matter to the caeca may also rise, and this may lead to an increase in microbial activity due to the additional availability of carbohydrate and protein for fermentation (Bedford, 1992; Annison, 1993). As a consequence, a build up of toxic secondary by-products from fermentation may occur, or colonies of toxigenic species such as *Clostridia* may cause the bird to be immuno-compromised, and thence lead to disease.

A schematic diagram representing the effects that NSP and viscosity can have on the bird is given in figure 1.5 below.

**FIGURE 1.5. POSSIBLE EFFECTS OF NSP ON NUTRITIONAL  
VARIABILITY OF CEREALS.**



#### 1.4. 6. Bird age

There are physiological factors that change the rate of ileal starch hydrolysis in the bird. Although broilers are only grown to slaughter weight during a short period of time (typically to seven weeks of age), bird age and maturity have been shown to affect enzyme production. Pancreatic enzymes and gall bladder secretions alter in response to dietary concentration of target molecules, and their activity increases with bird age. It has been suggested that this development of secretion limits digestion efficiency and subsequent broiler performance in young growing birds (Nitsan *et al.*, 1991). Bedford (1992) also linked broiler efficiency with age, commenting that young birds faced limitations in their ability to digest feed due to poor enzyme secretion; he highlighted the influence of viscous dietary components, which have a larger effect in broilers under four weeks of age. As efficiency of feed utilisation, growth rate, feed intake and the extent of influence of dietary anti-nutritive factors depend upon the age and maturity of the broiler chicken, it follows that bird age must be taken into account when using diets which potentially contain viscous compounds, or other nutrients that certain age groups may find difficult to digest (e.g. amylose). Digestibility studies using

wheat have shown that older broilers utilise feed more efficiently than young ones, and have fewer problems with anti-nutritive substances than younger animals as a result of improved enzyme secretion and increased ability to hydrolyse dietary components (Rogel *et al.*, 1987; McNab, 1993).

#### **1.4.7. Feed processing**

Wheat is not usually fed whole in poultry diets. It generally passes through certain types of processing, both before and after combination with other dietary components, in order to improve its nutrient digestibility and ease of handling. Whole feed wheat is milled to fracture the grains and expose the internal structures, e.g.; the starchy endosperm. As the wheat grains split the starch granules are broken open and the surface area of the endosperm is increased. Greater starch damage has been linked to improved digestibility (Bedford, 1992) as it increases the rate of digestion *in vivo* by increasing the availability of substrate for enzymatic attack.

The milled wheat will then be combined with other feed ingredients to give a diet that satisfies the nutrient requirements of the bird. Wheat based



diets may be fed to broilers in this meal form, though commercial diets are more usually subjected to other processes to make the feed more digestible (Rogel *et al.*, 1987) and easier to handle, particularly regarding dust reduction. Pelleting is a common processing method used with broiler rations in the UK. The feed is compressed, by forcing it through the holes of a die, into small pellets. Water may be added to assist the process where the feed has a high dry matter content (Larbier & Leclercq, 1994). Pelleting causes the feed to heat up (generally to about 70°C or above), which results in the gelatinisation of starch and denaturation of protein, improving the ease of digestion of these nutrients by making them easier to hydrolyse in the gut (Snow & O'Dea, 1981; Rogel *et al.*, 1987). Care needs to be taken, however, to ensure that the heat of the process does not cause increased solubilisation of undesirable NSP fractions, or the transformation of starch into its crystalline form (formed during cooling) which is often indigestible (Colonna *et al.*, 1992; Englyst, 1992; McNab, 1993). Pelleted feed may be crushed again into what is termed 'crumb' diets, for feeding to young birds that are too small to easily consume whole pellets (Larbier & Leclercq, 1994). Heat expansion and extrusion of diets acts on the nutrients in a similar way improving digestibility (Larbier & Leclercq, 1994).

## **1.5. THE EVALUATION OF THE NUTRITIVE VALUE OF WHEAT IN BROILER CHICKEN DIETS**

### **1.5.1. Laboratory Analysis**

The most common laboratory assessment of feed components including cereals, for inclusion in poultry diets is collectively known as 'proximate analysis'. This involves the measurement of moisture, protein, fat, fibre and inorganic matter contents. The amino acid, vitamin and mineral profile of a feedstuff can also be examined. Under ideal conditions the variability and quality of feed ingredients is analysed before it is included in a whole ration so as to ensure a nutritionally adequate, balanced diet for the animal. The determinations generally referred to as 'proximate analysis', are commonly assessed, particularly in finished or processed feeds, where such values have to be declared (Ball, 1988).

As discussed in section 1.2.3, different batches of wheat can show great variation in composition and quality. Apart from cursory visual assessment to assess spoilage or damage of the grain, it will usually have

been analysed for its dry matter and protein content. Currently there are few other analyses carried out on wheat destined for animal feed, as laboratory analysis is costly and often not deemed as important a factor as it is in the high quality, human food grade wheats used in baking.

In order to formulate broiler diets to match the level of nutrients required by the chicken without knowledge of the chemical composition of each batch of materials, standard values are used which have been calculated from analysis of samples of feedstuffs thought to be typical of their type. In a review investigating the financial and nutritional implications of variability and quality control of raw materials for animal feed, Ball (1988) described how there was no segregation of material with respect to its origin (variety, harvesting conditions) or quality. Obviously, in light of the standard values used, this may lead to over or under estimation of the nutrients contained within that material during feed formulation, which could consequentially affect the performance of the growing broiler. As faster and easier methods of quality control and assessment become available, such as the rapid near infra-red analysis, more accurate feed formulations will be possible.

Currently feed is analysed in detail only when there is a severe problem that has become evident during the growing period of the broilers, or during controlled research trials. As yet, information on the availability of the nutrients measured, and the resulting contribution to broiler chicken growth is not available.

### **1.5.2. Animal Feeding Experiments**

The nutritional quality of a poultry diet is of most commercial importance through the weight gain and efficiency of conversion of feed into tissue (FCR) of the bird. The most basic method of determining the quality of feedstuffs for poultry is by means of feeding experiments, where liveweight gain and feed intake can be measured accurately under controlled conditions. The FCR can then be calculated (feed intake divided by weight gain) to give an efficiency value for that feed.

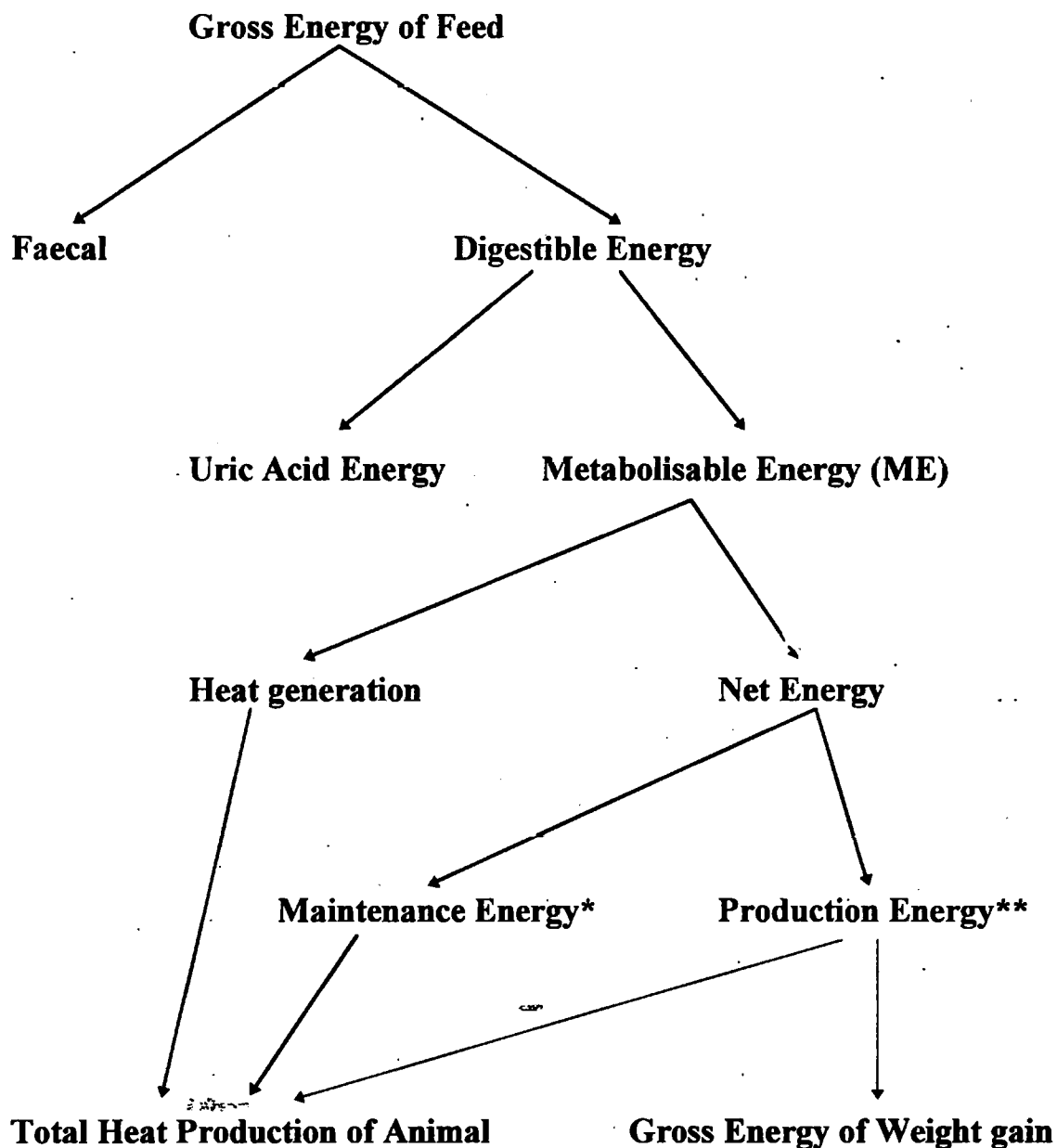
In this study these parameters were defined collectively as the 'productive performance' of the broilers. These evaluations are very specific

to the formulation and quality of the diet that the animal receives, and can vary considerably under commercial conditions due to disease exposure as well as dietary influences. As a measurement of dietary quality, productive performance is possibly the most valuable information for broiler growers.

### **1.5.3. Metabolisable Energy**

The metabolisable energy (ME) available to the animal is a standard measurement, calculated from animal experiments, used by animal nutritionists to gauge the nutritional value of the diet (McDonald *et al.*, 1981). It is a method of determining feeding quality under controlled conditions. The energy of the diet is partitioned out and utilised for different purposes within the animal (figure 1.6). ME is the dietary energy available for body maintenance and tissue growth, being the energy value of the nutrients that are digested and absorbed across the gut wall minus the energy lost in uric acid. There are two main methods of determining the ME of poultry diets.

**FIGURE 1.6. GENERAL PARTITIONING OF FEED ENERGY**



\* Energy required for maintaining body, e.g. respiration, gaseous exchange.

\*\* Energy retained for body growth.

From McDonald *et al.*, 1988.

## (i) Apparent metabolisable energy

The simplest way of measuring ME is by the Apparent Metabolisable Energy (AME) system. AME is calculated from data generated from experiments using young broilers (usually around 3 weeks of age) kept in cages under controlled conditions, fed *ad libitum*, and with the faeces collected over four days. The AME of the diet is calculated by determining the total amount of energy contained in the diet consumed by the bird, and the amount of energy excreted, and calculating the difference that is retained by the animal for growth and maintenance (Hill & Anderson, 1958; McDonald *et al.*, 1981). Equation 1.1 shows the calculation for AME.

### Equation 1.1. AME calculation:

$$\begin{array}{lcl} \text{AME} & = & \frac{(\text{Energy intake (MJ)} - \text{energy excreted (MJ)})}{\text{Feed intake (kg)}} \\ \text{(MJ/kg)} & & \end{array}$$

The AME calculation can be modified to take into account the amount of nitrogen metabolised by the bird from the diet, and the corresponding energy utilised for nitrogen assimilation (Hill & Anderson, 1958). This is

known as the nitrogen correction factor, the corresponding value being expressed as AMEn. The total nitrogen excreted by the bird is subtracted from the total nitrogen ingested to give a value for the total nitrogen retained. This figure is then multiplied by the amount of energy required for the assimilation of retained nitrogen into body tissues (34.39 MJ/kg, Hill & Anderson, 1958) and subtracted from the original AME result (equation 1.2) (McDonald *et al.*, 1981).

**Equation 1.2. Nitrogen-corrected AME calculation:**

$$\text{AMEn} = \frac{((\text{Ein(MJ)} - \text{Eex(MJ)} - (\text{retained N(kg)}) \times 34.39 \text{ MJ/kg}))}{\text{Feed intake (kg)}}$$

(MJ/kg)

**(ii) True metabolisable energy**

AME values vary with respect to feed intake. In order to improve the method of AME evaluation, Sibbald and other workers developed a new method. This method is called the True Metabolisable Energy (TME) determination. The method involves tube-feeding a chicken, usually an adult



cockerel, a known weight of the test feed (typically 50g milled cereal), thereby removing feed intake inaccuracies (Sibbald, 1975, 1985); the bird is starved prior to the tube-feeding for a minimum of 24 hours (Shires *et al.*, 1979) to remove all previous feed material from the digestive tract. After feeding, excreta is collected over the following 48 hours. Control birds are fed only pure carbohydrate (usually glucose or sucrose in solution), in the same controlled manner, and the amount of non-dietary energy lost from the gut by these birds is calculated as endogenous energy loss (EEL). TME is calculated (and corrected for endogenous energy losses and nitrogen retention) in the same way as AME (equation 1.3).

**Equation 1.3. Nitrogen-corrected true metabolisable energy calculation:**

$$\text{TME}_{\text{En}} = \frac{(\text{E}_{\text{in}}(\text{MJ}) - \text{E}_{\text{ex}}(\text{MJ})) + \text{EEL}(\text{MJ}) - (\text{ret. N}(\text{kg}) \times 34.39 \text{ MJ/kg})}{\text{Feed intake (kg)}} \text{ (MJ/kg)}$$

### (iii) Limitations of ME systems

The AME measurement is currently thought to have some deficiencies for determining the nutritional value of a diet for a number of reasons. The main limitation is that there is no account taken of the energy that is lost as heat or for maintenance metabolism. Special metabolism chambers can be used to measure respiration and heat generation, but these are costly and complex. There are problems associated with precise measurements of feed intake for the AME method of feed evaluation. The *ad libitum* feeding of the birds leads to inaccuracies in feed intake measurements, due to clogging of feed around the mouth and dropping feed on the floor, or into water drinkers (McNab, 1993). Open-tray faecal collection can also be problematic, as the faeces may be contaminated with dropped food, which will raise its energy content, or the actual collection/weighing method may result in losses of faecal material (Wiseman & Inborr, 1990; McNab, 1996). Volatile fatty acids, which contribute to the energy value of the faeces, will also be lost due to exposure to air over several days. *Ad libitum* feeding also requires the test feedstuff to be fed as a complete diet, and essential dietary inclusions

(proteins, fats) may interact or interfere with the dietary ME evaluation. AME values are also affected by level of feed intake. Decreases in feed intake cause directly proportional increases in faecal and urinary energy, and exert significant influence on, and cause variability in the final AME value (Sibbald, 1975). AME does not take into account the amount of energy that is lost in the form of mucous and enzyme production (the endogenous energy losses), which will have been formed from metabolism of the diet previously consumed by the bird, and that may not be part of the energy intake that is being measured. Losses of feed energy to gut microflora during hind gut fermentation processes are also not taken into account. All these factors cause errors in the final estimation of the AME value for the feed under examination. There is no official consensus as to the relationship between AME and the productive performance of broilers (Rose *et al.*, 1992; Wiseman, 1992; McNab, 1996; Rose & Bedford, 1995).

There are some difficulties associated with the TME method of feed evaluation, and its relationship to broiler performance. Although problems with feed intake variation are overcome, excreta collection difficulties remain.

There is a reliance on the ability of the researcher to detect regurgitated feed contamination of faecal samples (which would affect the energy content of the faeces) and eliminate that bird from the data. Also, the result is further compromised by the introduction of more, less easily identifiable, complications (McNab, 1996) caused by the stress of starvation. Starved birds have been shown to increase pancreatic secretion (Nir *et al.*, 1993), therefore the endogenous energy losses will be higher in starved birds than in those fed *ad libitum*. TME results may therefore be artificially high. Tube-feeding is very difficult to perform on young broilers. Therefore only adult birds, or birds at least four weeks old (Shires *et al.* 1980), are recommended for use. Feed performance differences between test feedstuffs may not exist in adult birds due to the increased digestive efficiency, so there is little comparison with young broiler performance or AME. Secondly, 48 hour starvation and tube-feeding is highly stressful to the bird. This stress may directly affect the digestive functions of the gut, leading to poor digestion of the feed under investigation, and therefore a poor resulting TME value.

#### **1.5.4. Previous Investigations into Broiler Performance and Methods of Evaluation.**

There is published work that has investigated the relationship between factors present in broiler diets and broiler growth, feeding efficiency and ME. Early research into the nutritive value of wheat as a poultry feed was done by March and Biely (1973). They compared different wheat varieties in chicken feeding trials and analysed the chemical and physical properties of the wheat samples. They found that there was no correlation between wheat variety and any physical or chemical property of the wheat. They concluded that AME was influenced by other factors of the wheat. Recent research into the nutritional quality of wheat has investigated the reliability of AME trials, and it has been reported that AME differs between wheat variety. Wiseman (1993) found that low AME values were associated with reduced starch and dry matter digestibilities of the diet and that digestion of starch may therefore vary with respect to wheat variety. Wiseman (1993) also reported a negative correlation between AME and the amount of NSP in the wheat. Similar findings have been reported by other workers. Annison & Johnson (1989),

Wiseman & Inborr (1990) and Annison (1993) have all linked cereal NSP with decreased AME values.

Rose *et al.* (1992) found no differences in AME between wheat varieties, but found significant differences between variety for broiler weight gain and feed intake. This would suggest that the standard methods of ME evaluation were not directly related to the growth of the animal, and could not be used as a measure of nutritional quality of the wheat.

McNab (1991) found correlations between wheat density and the TMEn value of wheat for poultry, though there was little variation in TMEn between varieties and growing site of wheat used in the diets.

As yet there are no *in vitro* laboratory analyses that correlate well with broiler productive performance (Wiseman, 1990; Dale, 1994).

## 1.6. PROJECT OBJECTIVES

The specific objectives were:

1. To establish if the previously observed differences in growth of broiler chickens fed different wheat varieties were repeatable, and if these were evident between different harvest years.
2. To determine whether the growth differences of broiler chickens fed different wheat varieties were independent of the age of the bird.
3. To test whether differences in growth of broilers correlated to differences in the chemical composition, grain quality or energy availability of the wheat samples fed to the birds.
4. To determine whether varietal differences in the rate of the hydrolysis of wheat starch was correlated to differences observed in broiler growth when fed different varieties of wheat.

5. To establish whether differences in *in vitro* rate of starch hydrolysis, that were found to be associated with nutritional and growth characteristics of the original wheat samples, could be used to predict the productive performance of broilers fed other wheat varieties.



## **2. GENERAL MATERIALS AND METHODS**

### **2.1. WHEAT SAMPLES**

#### **2.1.1. Variety Selection**

The two feed wheat varieties predominantly used in this project were Dean and Beaver. These varieties were selected for use in the project because of their large differences in nutritional quality, as reported by Rose *et al.* (1992) who found that, out of six UK varieties from the harvest years 1990 and 1991 tested in broiler growth experiments, Beaver gave the poorest, and Dean the best, broiler performances.

The varieties selected had certain physiological differences. Beaver is a high yielding, soft feed wheat, with a typical yield of 9.70 t/ha. Dean is a lower yielding hard feed wheat variety, with a typical yield of 8.80 t/ha.

### **2.1.2. Harvest Years**

Experiments were conducted in the course of the project to test whether the nutritional differences were evident between different harvest years for these varieties. Dean and Beaver samples taken from three harvest years (1990, 1991 and 1992) were used in broiler feeding experiments and laboratory analyses. The year of harvest was used as a treatment factor in the statistical analysis of the results.

The wheat samples used in the final experiments of this study were the six varieties Dean, Beaver, Brigadier, Rialto, Riband and Haven and were harvested in 1994.

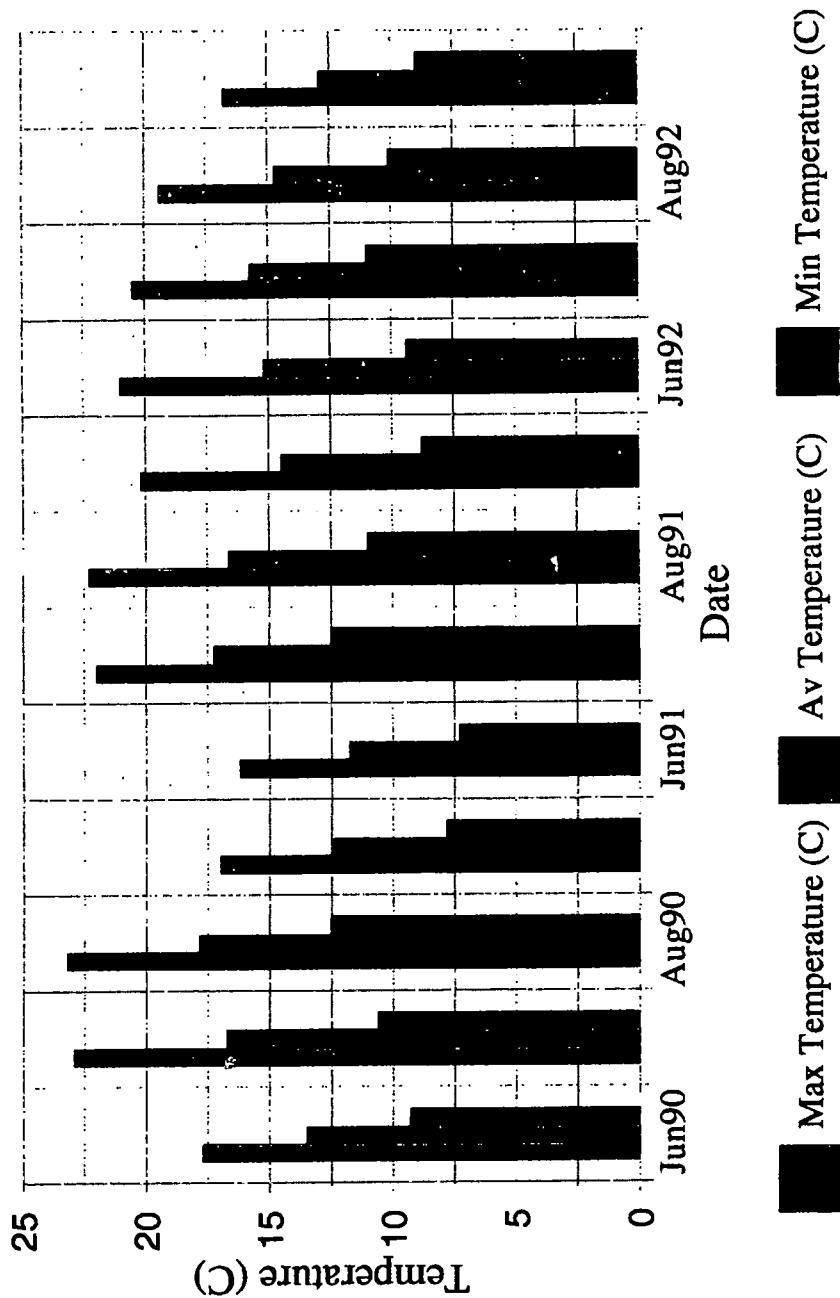
The meteorological characteristics (rainfall and average temperature) of each of the three years during the months of ripening and harvest were used to categorise the growing conditions, and to determine the effect of this on grain quality and development. The information was recorded at Harper Adams weather station for the months of June, July, August and September, and is shown in graphs 2.1 and 2.2 below.

The temperature during the ripening and harvesting months followed similar patterns in 1990 and 1991, but in 1992 the temperature was lower during the months of June, July and August. Rainfall during August 1992 was markedly higher than in any of the other periods recorded.

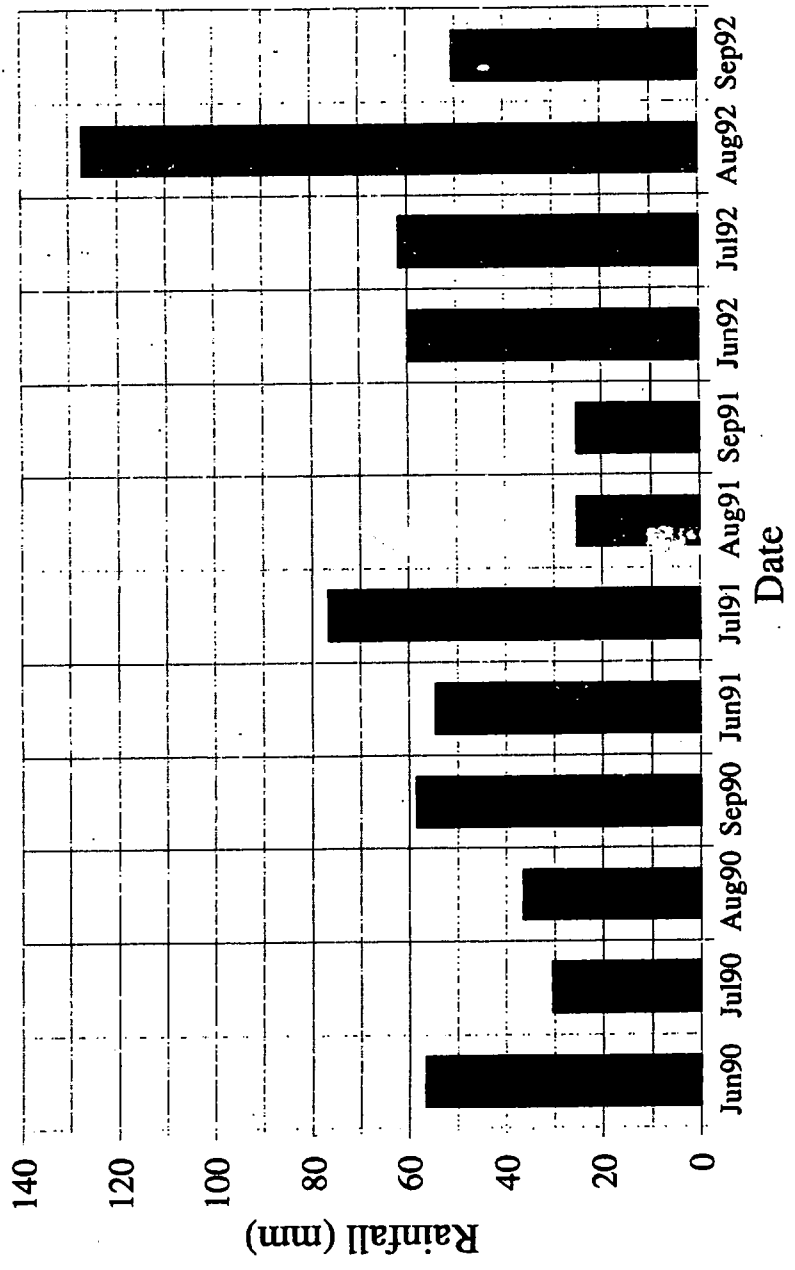
### **2.1.3. Growing Sites**

Each of the growing sites used was split into three land blocks, with the varieties sown in a randomised design within each blocks. This was done to reduce the effects of soil variation within the fields on grain development. Samples from 1990 were, in addition, grown at two different sites to further ensure representative samples. One site was at Escrick Park in Humberside, and the other at Hornsea in Yorkshire. Samples from 1991, 1992 and 1994 were produced on sites at Harper Adams. All the crops used in the project were treated with one application of fertiliser in April.

**GRAPH 2.1. TEMPERATURE DURING THE SUMMER MONTHS OF 1990, 1991 AND 1992**



GRAPH 2.2. RAINFALL DURING THE SUMMER MONTHS OF 1990, 1991 AND 1992



## **2.2. DIET PREPARATION AND FORMULATION**

### **2.2.1. Wheat Sample Preparation**

Stored samples of wheat from each of the randomised field plots harvested in 1990, 1991 and 1992 were sorted by variety and then mixed in equal proportions in order to give six pooled samples of Dean and Beaver from each of the three harvest years. The six variety samples from 1994 harvest, used in the final experiment, were also a mixture of grain from randomised field plots. In the experiment investigating effect of broiler age, diets were formulated with wheat harvested only in 1991 and 1992, due to depleted stocks of 1990 sample. The wheat samples were mixed in a horizontal mixer and hammer milled through a 4mm screen. Freshly milled wheat samples were used for each feeding experiment to avoid spoilage, and a sample was retained from each new batch of flour for laboratory analysis.

### **2.2.2. Diet Formulation**

The same diet formulation was used throughout the project, ensuring that the only factor varying between diets was the wheat sample. Each

milled wheat sample was mixed with the other feed ingredients in the proportions shown in table 2.1 using a horizontal ribbon mixer. The composition of the other ingredients was calculated from standard information reported by the individual manufacturers, and is given in table 2.2. Diets were prepared with 700 g/kg wheat inclusion, and each feeding trial, with the exception of the final one, was conducted using the prepared diets in basic meal form, thereby avoiding potential differences in digestibility that could be caused by further processing techniques.

**TABLE 2.1. EXPERIMENTAL DIET FORMULATION**

Ingredient	Proportion (g/kg)
Ground wheat	700
Full fat soya	155
Fish meal	97.5
Meat and bone meal	25
Methionine	2.5
Vitamin and mineral premix*	20

\*1g of premix provided, per kg of whole diet: vitamin A 40000 i.u./kg, vitamin D<sub>3</sub> 7500 i.u./kg, vitamin E 62.5 i.u./kg, amprolium 6.25 mg/kg, ethopabate 0.4 mg/kg and avoparcin 0.5 mg/kg.



**TABLE 2.2. CALCULATED COMPOSITION**

Nutrient	Quantity
Gross energy (MJ/kg)	16.5
Calculated ME (MJ/kg)*	12.3
Crude protein (g/kg)	215
Lysine (g/kg)	12
Methionine + cystine (g/kg)	9
Calcium (g/kg)	11
Phosphorus (g/kg)	7
Sodium (g/kg)	3

\* Calculated from values quoted in Novus, 1992.

### **3. CHEMICAL AND QUALITY ANALYSIS OF WHEAT**

#### **SAMPLES**

#### **3.1. CHEMICAL COMPOSITION**

The gross chemical composition of each of the wheat samples was measured by standard methods commonly used in the description of feedstuffs. The data was statistically analysed to determine significant differences between the wheat samples, due either to variety or harvest year.

##### **3.1.1. Materials And Methods**

Dry matter, crude protein, crude fibre and oil (as ether extract) were all determined according to AOAC procedure numbers 925.10, 984.13, 962.09, 920.39 respectively.

### **3.1.2. Statistical Analysis**

Each sample was analysed in four separate experiments conducted over a period of time, and all data was statistically compared in a two-way analysis of variance, with wheat variety and harvest year as treatment factors. Replication over time was used as a blocking factor.

### **3.1.3. Results And Discussion**

The results from the proximate analysis of wheat samples are given in table 3.1 below, and displayed in graph 3.1. All the values were within or close to the range of figures for these constituents quoted by Novus in their Global Feedstuffs data (1992), which are shown at the bottom of the table to aid comparison. The Novus data was compiled from standard references for the average feeding value and composition of feedstuffs world-wide (Novus, 1992).

Samples of Dean contained significantly higher levels of crude protein (5%) than Beaver for all three harvest years ( $p < 0.001$ ), with the 1992 samples containing the highest and the 1991 the lowest amounts. Varietal differences in protein between Dean and Beaver were 11% for

1990 samples, 4% for 1991 samples and 2% for 1992 samples. Variation in protein content was significantly different for the harvest years ( $p < 0.001$ ), and the change in percentage difference over harvest year was reflected in a highly significant variety x year interaction ( $p < 0.001$ ).

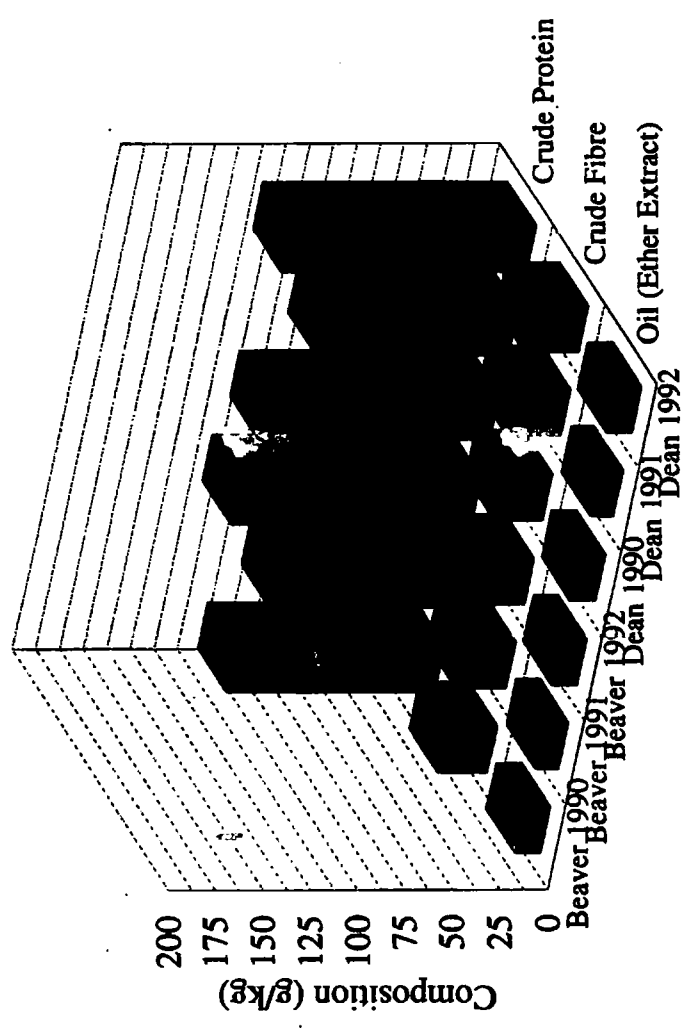
There was a significant difference ( $p < 0.05$ ) between harvest years for crude fibre content as a result of the high fibre content recorded for the 1992 samples of Beaver and the low measurement for Dean 1990. The result obtained for Beaver 1992 may have been a consequence of the high rainfall and low temperatures during the grain filling period of the growing season, although Dean was not affected in the same way. The implication of this observation was that the varieties responded physiologically to environmental stresses in different ways, resulting in differences in chemical composition between samples of the same genotype but grown under varying climatic conditions (Wiseman, 1990). The oil contents of both varieties were similar, lower than the Novus standard figure, and unaffected by harvest year. There were no other significant differences or interactions for chemical composition between the two varieties from these three harvest years.

**TABLE 3.1 PROXIMATE ANALYSIS OF WHEAT SAMPLES FROM 1990, 1991 & 1992**

Variety	Year	Dry matter (g/kg)	Crude Fibre (g/kg)	Crude Protein (g/kg)	Oil (g/kg)
Beaver	1990	881	26	110	13
	1991	879	26	97	12
	1992	883	32	127	13
Mean		881	28	111	13
Dean	1990	877	25	122	13
	1991	878	28	101	12
	1992	881	28	129	13
Mean		879	27	117	13
SEM variety		2.0	0.8	0.3***	0.4
SEM year		2.4	1.0*	0.4***	0.5
SEM variety x year		3.4	1.4	0.6***	0.7
Residual DF		15	15	15	15
Novus range (Novus, 1992)		856 - 897	18 - 43	103 - 145	15 - 23

\*p<0.05; \*\*p<0.01; \*\*\*p<0.001. NB Oil measured as ether extract

**GRAPH 3.1. COMPARISON OF THE CHEMICAL COMPOSITION OF DEAN AND BEAVER WHEAT SAMPLES HARVESTED IN 1990, 1991 AND 1992.**



The experiments conducted by Rose *et al.* (1992) gave values in chemical composition for UK wheat varieties that were comparable to the results from this experiment. The significant protein differences between the two varieties were similar to those recorded for Canadian wheat samples by Schollenberger & Curtis (1949) and March & Biely (1973) during their investigations of the variability in chemical composition of feed wheat. Their results had also indicated large variation in protein content between genotypes. Nichol *et al.* (1993) also reported significant variation in protein level between four UK wheat varieties used in broiler chick experiments.

## **3.2. GRAIN QUALITY**

The physiology and quality of the samples were analysed using methods originally devised by the baking industry. The results obtained from these experiments gave information on the grain physiology, natural enzyme activity and gelatinisation properties of the varieties.

### **3.2.1. Materials And Methods**

Hagberg falling number was determined using AOAC method number 976.13. Grain hardness was measured by Near Infra-red Reflectance (NIR) according to AOAC method number 989.03, with the softest (Beaver 1992) wheat sample as the zero calibration point. Specific weight of the grain was analysed using a chondrometer, whereby the weight of one hectolitre of grain was measured. Particle size was measured at Weston Research Laboratories by use of a Sympatec laser particle sizer, and the proportion of A- to B-type granules was estimated from this data by the percentage of particles less than 11  $\mu\text{m}$ , as discussed by Parker (1985) who categorised starch granules less than 10  $\mu\text{m}$  to be B- and C-types, and those between 10 - 50  $\mu\text{m}$  to be A-types.



### **3.2.2. Statistical Analysis**

Each sample was analysed with replication over time, with the exception of particle size. Hagberg falling number, hardness and specific gravity data were statistically compared in a two-way analysis of variance, with wheat variety and harvest year as treatment factors. Particle size could not be statistically analysed as there was no replication of this measurement.

### **3.2.3. Results And Discussion**

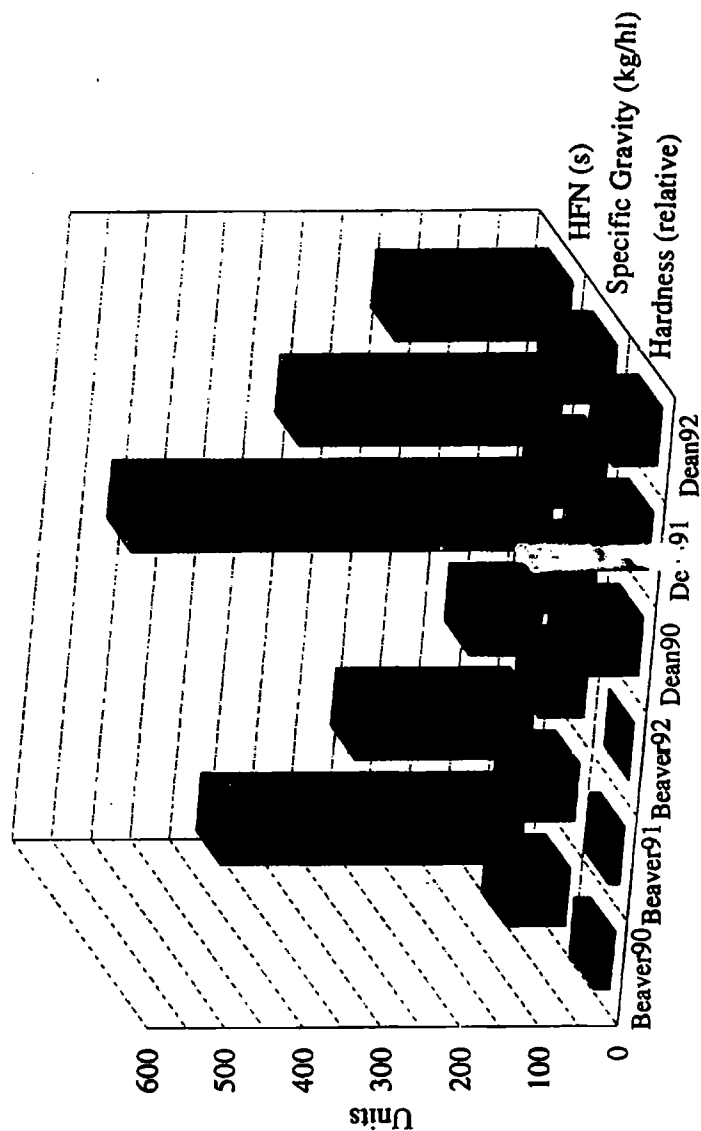
The results of the grain quality analysis are given in table 3.2 and graphs 3.2 and 3.3 below. Values for HFN, hardness and specific gravity declined over the three growing years, suggesting that the quality of the grain also declined over this period. Significant differences and interactions for these measurements were found between wheat varieties and harvest years for the analysed data.

**TABLE 3.2. QUALITY ANALYSIS OF WHEAT SAMPLES 1990 - 1992**

Variety	Year	Hagberg falling number (seconds)	Specific gravity (kg/hl)	Endosperm hardness (relative units)	Particle size (%<11 µm)
Beaver	1990	381	74.2	20.30	12.7
	1991	221	71.9	13.00	14.87
	1992	89	77.7	0.00	14.26
	Mean	230	77.9	11.10	13.94
Dean	1990	532	71.9	86.63	2.29
	1991	328	72.6	75.43	3.04
	1992	218	65.1	56.30	6.78
	Mean	359	70.2	72.79	4.04
SEM Variety		3.2***	0.22***	0.832***	-
SEM Year		3.9***	0.26***	1.008***	-
SEM Variety x year		5.5**	0.36***	1.426*	-
Residual DF		15	15	15	-

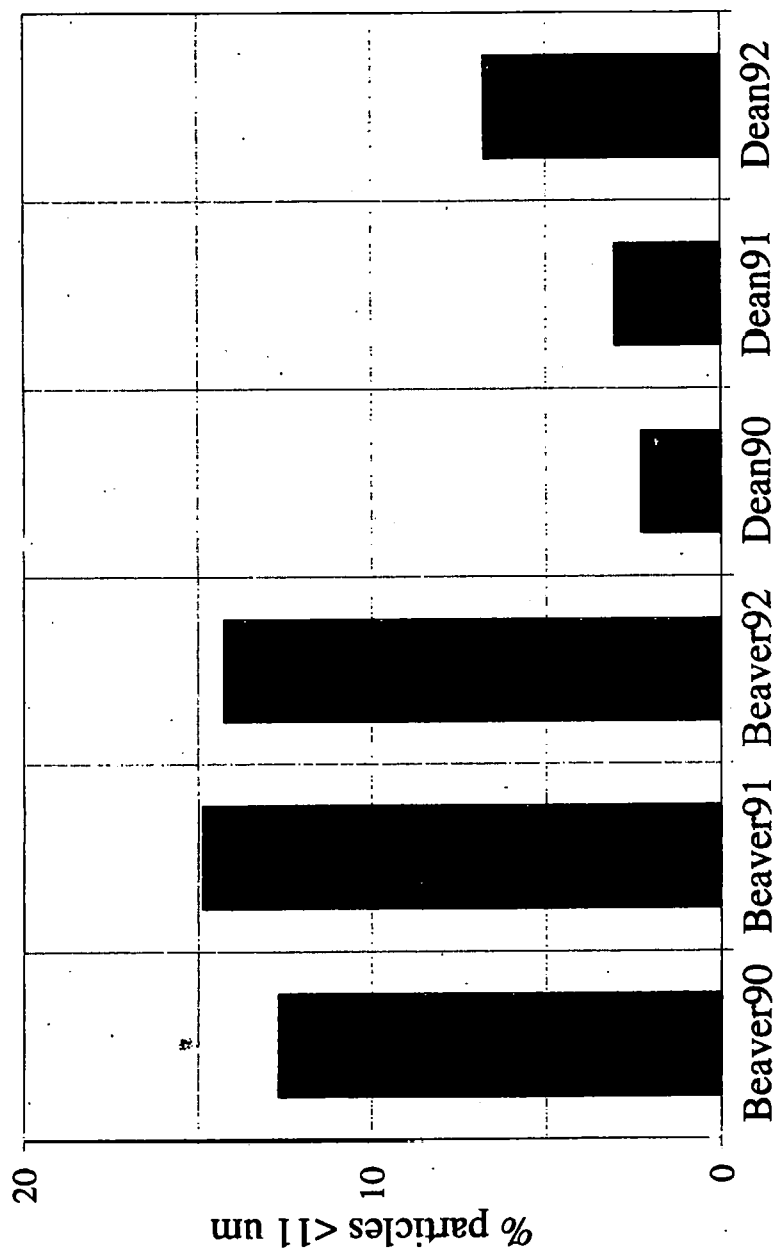
\*p<0.05; \*\*p<0.01; \*\*\*p<0.001

**GRAPH 3.2. COMPARISON OF BAKING QUALITY CHARACTERISTICS OF DEAN AND BEAVER WHEAT  
SAMPLES HARVESTED IN 1990, 1991 AND 1992.**



**GRAPH 3.3. COMPARISON OF PARTICLE SIZES OF MILLED DEAN AND BEAVER WHEAT SAMPLES**

**HARVESTED IN 1990, 1991 AND 1992.**



Dean had a consistently higher (on average 50%) Hagberg falling number, suggesting that grain sprouting incidence was very low and that the endospermic starch in this variety was more readily gelatinised than that of Beaver. This might have been an effect of the higher proportion of large starch granules (Gruppen, 1996), as indicated by the particle size data, where the results showed that there were over three times more small (<11 µm diameter) particles in Beaver than in Dean (Bechtel *et al.*, 1993). Therefore, it may be suggested that Dean had proportionally less small, B- or C-type granules than Beaver, although this would require clarification by electron microscopy methods which were not available. It may also indicate that there are more 'fines' (very small, dusty particles) produced in meal diets formulated with the softer variety (Larbier & Leclercq, 1994; Vincent *et al.*, 1995). Increased fines in the diet can cause low feed intakes due to clogging around the mouth, and may also inhibit crop emptying, by the formation of sticky, doughy lumps of feed that get lodged in the crop and are difficult to break down. Certainly the texture (hardness) of the grains could be linked to the particle sizes, with the hard wheat, Dean, producing larger fragments due to its cracking during milling, in contrast to the 'squashing' effect seen when milling soft wheats such as Beaver (Vincent *et al.*, 1995).

The significant differences in hardness of endosperm texture may be a reflection of the higher protein content observed for samples of Dean during the proximate analysis of the grain (Sulaiman *et al.*, 1994). Regression analysis did not detect any simple linear relationship between these two measurements. This was probably due to variations in the distribution of protein between the internal grain structures (e.g. between embryo and endosperm), measurement of which was outside the scope of this investigation.

Grains of Dean were better filled, and contained starch that was possibly more soluble and probably packed into larger, A-type granules (which are generally considered to be more easy to digest than B-type granules (Wiseman, 1990; Gruppen, 1996), see section 1.4.1). The harder Dean grains, when ground, produced meal with more large particles than the soft, more crushable Beaver, which might influence the palatability for broilers and thence impact on feed intake (Wiseman, 1990). The results of the experiment indicated that Dean had potentially higher feeding value than Beaver.

### **3.3. CARBOHYDRATE ANALYSIS**

The objective of these experiments was to quantify the amounts and types of carbohydrates, other than the crude fibre assayed in the proximate analyses, within the wheat samples Dean and Beaver from 1990, 1991 and 1992 harvest years.

#### **3.3.1. Materials And Methods**

##### **(i) Non-starch polysaccharides**

The NSP profile of each wheat sample was classified and analysed in accordance with a method devised by Dr Hans Englyst at the Dunn Clinical Nutritional Centre (Englyst *et al.*, 1992). The methodology is summarised in appendix 1. The classifications used were:

- (a) Total Dietary Fibre
- (b) Insoluble Dietary Fibre
- (c) Soluble Dietary Fibre ((a) - (b))

The procedure used was an enzymic-chemical method, whereby fat-free wheat starch was liberated from its granular structure by gelatinisation in water and DMSO (a wetting agent), and removed by digestion with amylase, pullulanase and pancreatin. The insoluble NSP was isolated by the repeated washing of the whole, enzymically-treated sample with distilled water. Sulphuric acid hydrolysed the NSP portion to neutral sugars and uronic acid, which were measured by colorimetry.

#### **(ii) Total starch and free glucose**

The total starch and free glucose content of each of the wheat samples was determined using the method devised by Englyst (Englyst *et al.*, 1992). This method allowed classification of the glucose and starch according to its digestibility and availability to the animal. The method used in this instance was one which simulated the digestive processes as they occur in monogastric animals, and is summarised in appendix 2. The wheat sample was finely ground, suspended in buffer and gelatinised by boiling. It was incubated with invertase, in order to convert all free and soluble simple sugars to glucose. A sub-sample was taken from the mixture, quenched in ethanol to stop the enzymic activity, and the amount of glucose present assayed colorimetrically. This portion represented the



amount of freely available glucose in the wheat sample. The remaining reaction mixture was then exposed to enzymic attack by amyloglucosidase, invertase and termamyl, and further degraded by application of strong alkali, and alternate boiling and freezing. These measures ensured that all the starch present within the wheat sample was reduced to basic glucose subunits. The amount of glucose present within the reaction mixture was then assayed in the same way as for the determination of free glucose. To calculate the amount of starch that had been hydrolysed, the amount of total free glucose in each sample was subtracted from the total glucose.

### **(iii) Amylose: amylopectin ratio**

The ratio of amylose to amylopectin in the endospermic starch of Dean and Beaver- from 1990, 1991 and 1992 harvest years was determined colorimetrically by a procedure devised by Jarvis & Walker (1993) (appendix 3). The wheat samples were treated with ethanol, to remove lipids, and then dissolved in potassium hydroxide and distilled water. A small sub-sample of this solution was neutralised with hydrochloric acid and stained with iodine solution. The samples were then analysed spectrophotometrically, and compared against amylose and

amylopectin standard absorbances in a series of simultaneous equations to calculate the comparative amounts of amylose and amylopectin in the sample.

### **3.3.2. Statistical Analysis**

Each sample was analysed as four time replicates for NSP and amylose:amylopectin ratio. Ten replicate analyses were used for the assessment of total starch and free glucose. Wheat variety and harvest year were used as treatment factors in the analysis of variance, and the data was blocked over time.

### **3.3.3. Results and Discussion**

The results of the carbohydrate analysis are shown in table 3.3 and figures. There were significant varietal differences for the free glucose content and amylose:amylopectin ratio ( $p < 0.001$ ) of the wheat samples. Beaver had consistently higher levels of free glucose than Dean, which may have been related to the lower values of Hagberg

**TABLE 3.3. CARBOHYDRATE ANALYSIS OF DEAN AND BEAVER WHEAT HARVESTED IN 1990, 1991**

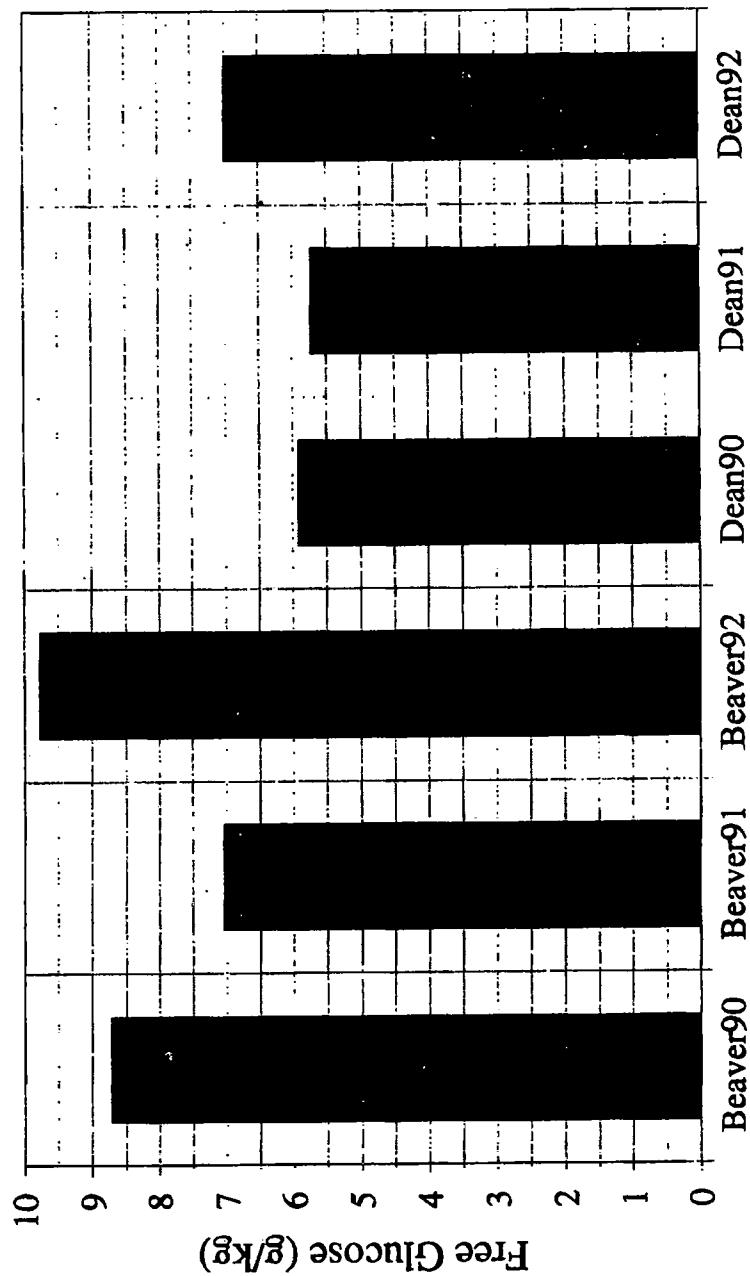
**AND 1992.**

Variety	Year	Total Starch (g/kg)	Free glucose (g/kg)	Total NSP (g/kg)	Insoluble NSP (g/kg)	Soluble NSP (g/kg)	Am:Ap ratio
Beaver	1990	640	8.7	136	98	39	0.595
	1991	652	7.1	136	111	25	0.628
	1992	606	9.8	134	97	37	0.557
Mean		632	8.5	135	102	33	0.594
Dean	1990	636	5.9	134	100	34	0.466
	1991	660	5.7	132	107	25	0.485
	1992	604	7.0	142	99	44	0.460
Mean		634	6.2	136	102	34	0.470
SEM Variety		3.0	0.11***	1.8	1.6	1.8	0.0151***
SEM Year		3.7***	0.14***	2.2	2.0**	2.2**	0.0185
SEM Variety x year		5.2	0.20***	3.1	2.9	3.1	0.0262
Residual DF		40	40	14	14	14	15

\*\*p<0.01; \*\*\*p<0.001

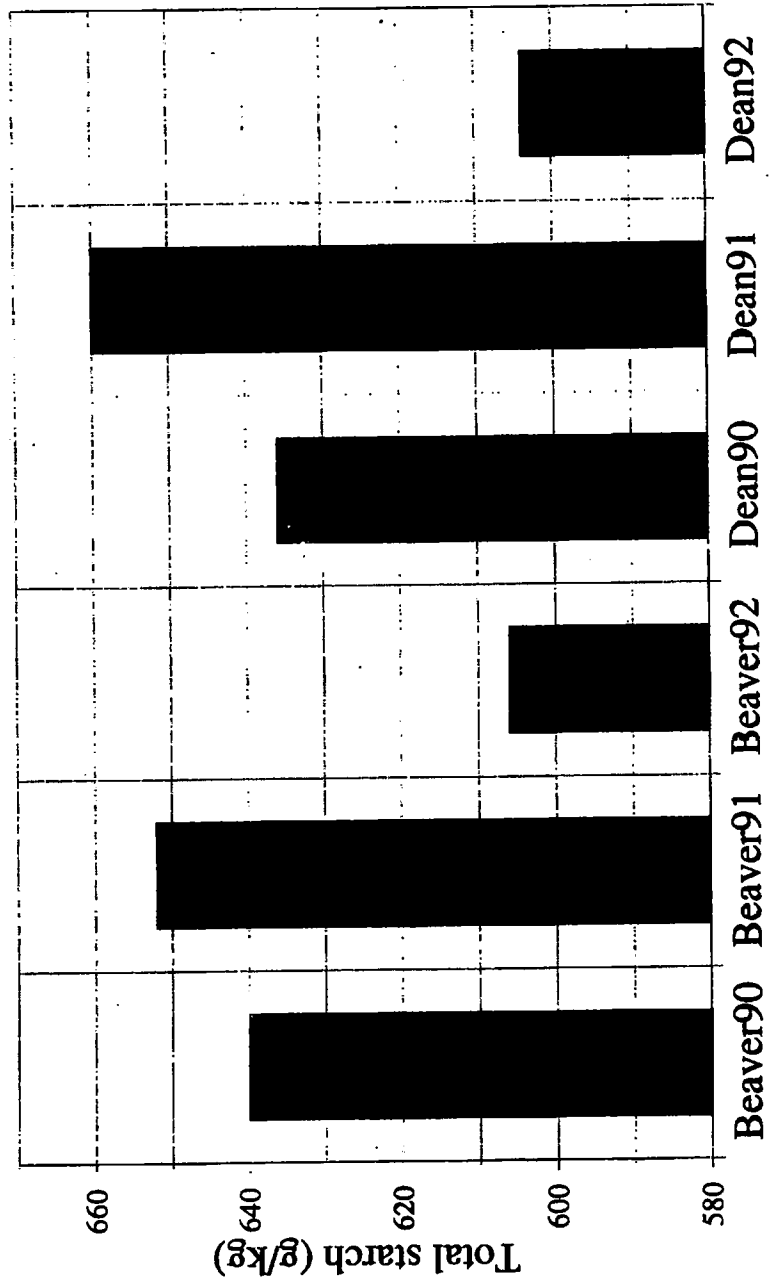
**GRAPH 3.4. FREE GLUCOSE CONTENT OF DEAN AND BEAVER WHEAT SAMPLES HARVESTED IN**

**1990, 1991 AND 1992**

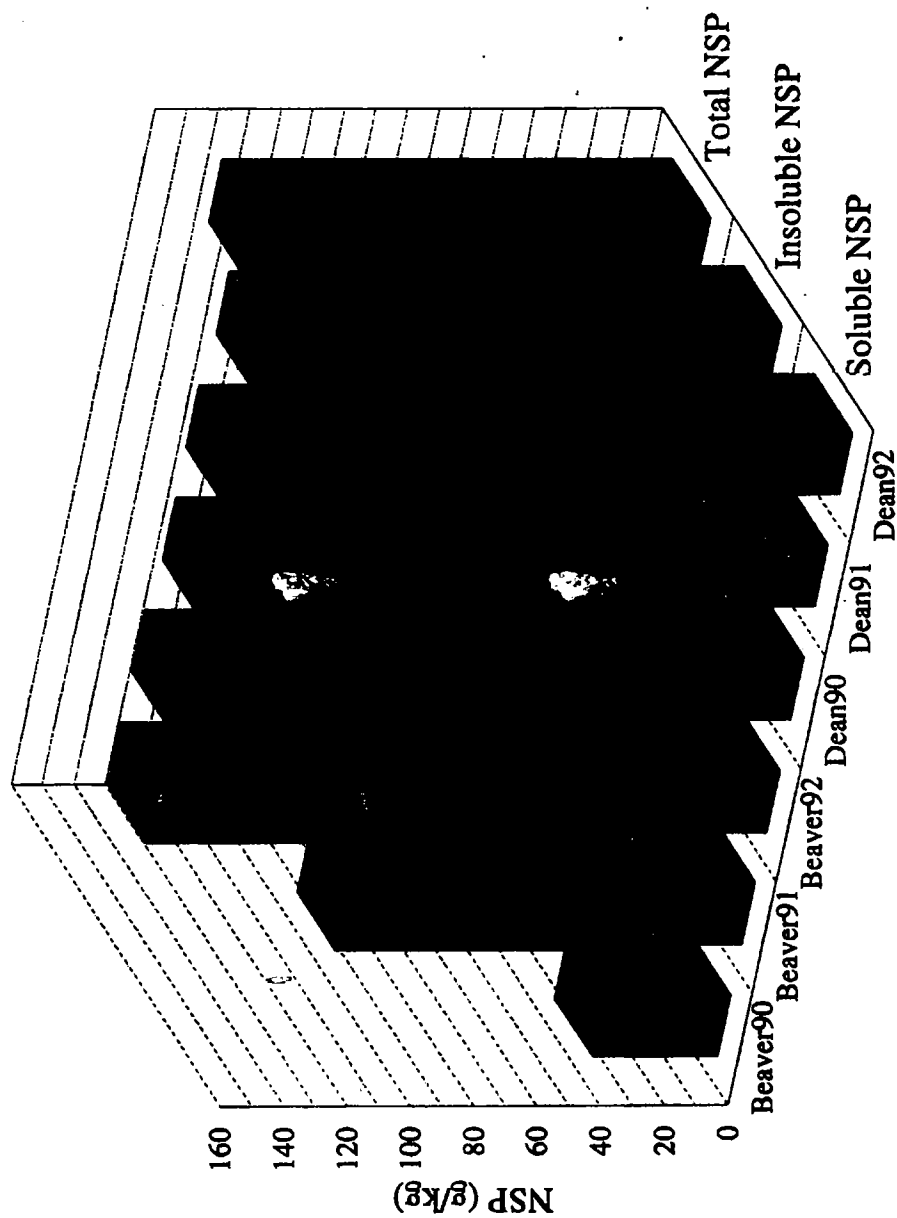


**GRAPH 3.5. TOTAL STARCH CONTENT OF DEAN AND BEAVER WHEAT SAMPLES HARVESTED IN**

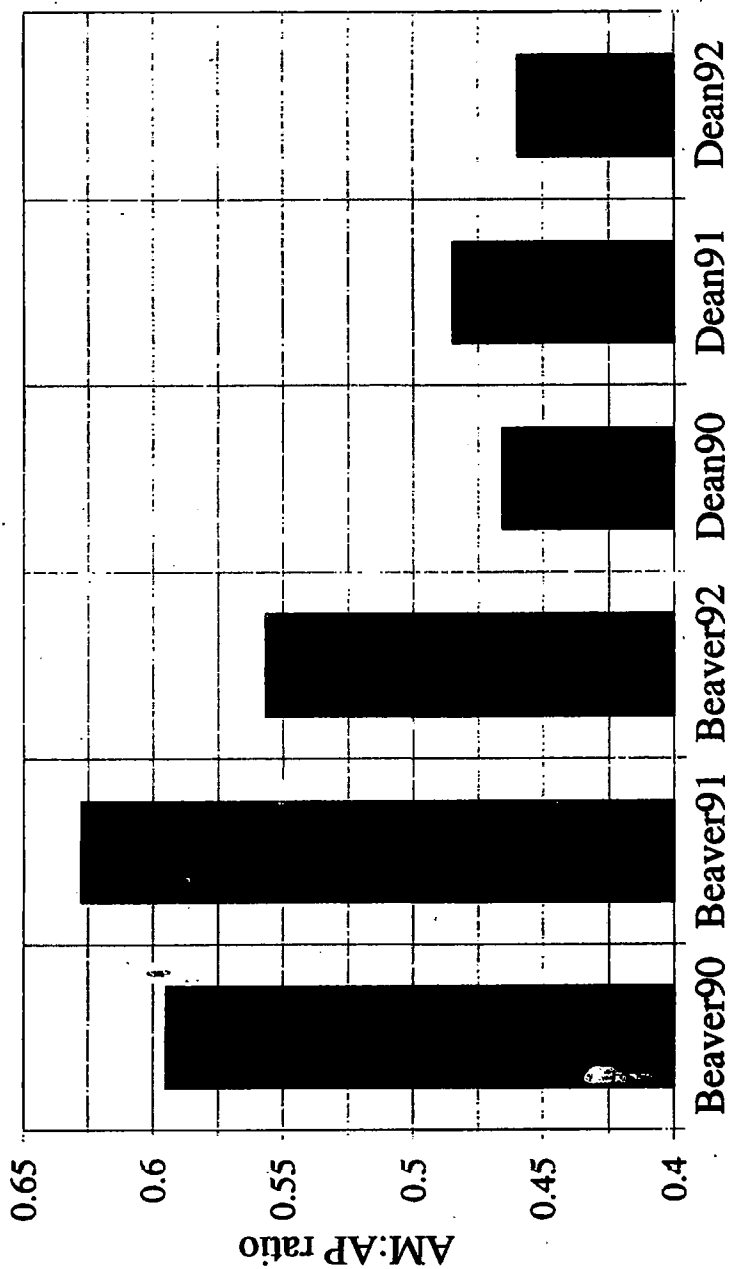
**1990, 1991 AND 1992**



**GRAPH 3.6. NON-STARCH POLYSACCHARIDE CONTENT OF DEAN AND BEAVER WHEAT SAMPLES HARVESTED IN 1990, 1991 AND 1992**



**GRAPH 3.7. AMYLOSE TO AMYLOPECTIN RATIOS OF DEAN AND BEAVER WHEAT SAMPLES  
HARVESTED IN 1990, 1991 AND 1992**



falling number obtained for Beaver in the grain quality analysis. Low values for HFN indicated higher  $\alpha$ -amylase activity (Warchalewski *et al.*, 1989; Wiseman & Inborr, 1990), i.e. that more  $\alpha$ -amylase enzyme had been released from the aleurone layer of the grain for catalysis of endospermic starch to limit dextrins and thence to glucose subunits (Gruppen, 1996). Wheat grains with more  $\alpha$ -amylase activity resulted in lower HFN because the action of the enzyme caused increased liquefaction of the endospermic starch (by loss of gelatinisation properties due to hydrolysis of the available starch to glucose) which decreased the viscosity of the gelatinised wheat solution (Wiseman, 1990; Best & Muller, 1991). The overall effect of this was seen as a decrease in the amount of time needed for the plungers to drop through the solution and, hence, a lower (faster) falling number was recorded. As Beaver had the lowest HFN, it probably had the highest amylase activity, which resulted in higher levels of free glucose in the wheat grain.

Total starch content was significantly different between harvest years. For both varieties 1991 gave the highest and 1992 the lowest values of total glucose. The amount of total starch in the six wheat samples was within the range (504-689 g/kg) previously reported for feed-grade wheats



used in broiler diets (Snow & O'Dea, 1981; Rogel *et al.*, 1987; Annison, 1990; Nichol *et al.*, 1993). Longstaff & McNab (1986) and McNab (1991) had previously reported differences in the starch content of wheat due to environmental factors. The drier weather conditions of 1990 may have had a slightly negative effect on the amount of starch laid down by reducing amylose synthesis (Jenner *et al.*, 1991). However, it appeared that the grain was even more adversely affected in 1992, when temperature was low and rainfall was high. Both Dean and Beaver appeared to respond to climatic effects in the same way with respect to starch synthesis, however there was not enough environmental information available to speculate on the causes of these differences. Nichol *et al.* (1993) had previously reported significant differences in starch content between four UK wheat varieties. Although the total starch content of the samples of Dean and Beaver was within the range (533-670 g/kg) quoted by Nichol *et al.* (1993), the range was smaller and genotypic differences were not found during this experiment.

There was a trend linking free glucose to total starch content, where the wheat with the lower amounts of total starch (1992 samples) tended to have the higher amounts of free glucose. This might have been an effect of the higher rainfall during final ripening and lower temperatures in that

year, which could have given rise to poorer grain filling and higher  $\alpha$ -amylase activity in relation to the other two years (Wiseman, 1990), as also observed in the HFN data. The variety x year interaction for free glucose may have been a reflection of the different ways that the two varieties responded to climatic pressures over the three years. Dean showed a similar level of free glucose in both 1990 and 1991, and only an increase in 1992, whereas Beaver gave different levels for all three years, with 1991 being the lowest and 1992 the highest. There are many aspects of the environment that interact with variety to produce such differences, and the only information available regarding these samples was temperature and rainfall data, so it was only possible to tentatively speculate how these two parameters might have played a role in producing the differences in free glucose content. Dean may have been responding more to the temperature than Beaver, in that its free glucose content in the grain was similar in years when temperature was of a consistently higher level (average 17 - 18 °C in July and August of 1990 and 1991), and increased when temperature dropped to an average of 15 °C (as in 1992).

There were no significant differences between wheat varieties for total, insoluble or soluble NSP fractions. The average value of NSP

fraction for all wheat samples was 135.7 g/kg total NSP, 101.8 g/kg insoluble NSP and 33.8 g/kg soluble NSP. The values were similar to those reported by Nichol *et al.* (1993) who found total NSP to range between 138-144 g/kg for their four UK wheat varieties. There were significant differences between harvest year for insoluble NSP, which was also reflected in the soluble NSP fraction. This was because the soluble fraction was calculated as the total NSP minus the insoluble NSP, and the harvest year differences in the insoluble fraction were therefore carried over into the soluble NSP result. The reason for the significant year effects was the marked rise in the insoluble NSP content of 1991 wheat samples. These samples had an average of 10g/kg more insoluble NSP compared to the 1990 and 1992 samples, and this may be the reason why higher levels of total starch (which possibly included some of this NSP fraction as a result of chemical degradation methods used to extract all the starch after removal of free glucose) were recorded in the 1991 samples. The insoluble NSP fraction is comprised of cellulose and long chain structural carbohydrates, often referred to in nutritional studies as indigestible fibre (Englyst *et al.*, 1992). These compounds are laid down in the grain for drought resistance (Dixon, 1985), and, even though temperatures were similar to 1990, the markedly lower rainfall during

August and September in 1991 most likely caused the observed increase in NSP content of the wheat samples.

The chemical nature of the starch was investigated by measuring the amylose to amylopectin ratio present within the granules of the endosperm, and was found to be significantly different between the two varieties ( $p < 0.001$ ). The grand mean ratio of the two starch polymers was 0.532, which was higher than the typical value of 0.430 for cereal starches (Morrison, 1992). However this value is thought to vary considerably, and high amylose cereal variants may show much higher ratios (Morrison, 1992). Dean had, on average, a 21% lower ratio than Beaver, and therefore proportionally more amylopectin. This could have been linked to the types of starch granules present in the endosperm, as A-type granules are thought to contain more amylopectin and have a more flattened, larger surface area than B- or C-type granules (Morell *et al.*, 1995), and the results of this, and the grain quality experiments, indicated that Dean was likely to have a larger proportion of A-type granules than Beaver. The starch in Dean would probably be more readily solubilised in the gut, and more available for digestion when fed to broiler chickens.

## **4. BROILER FEEDING EXPERIMENTS**

### **4.1. NUTRITIONAL COMPARISON OF DEAN AND BEAVER USING 7-21 DAY OLD BROILERS**

#### **4.1.1. Objectives**

The objective of this experiment was to determine any differences in the weight gain, feed intake, feed conversion ratio and AMEn in 7-21 day old broilers fed diets containing two wheat varieties, Dean and Beaver, grown in 1990, 1991 and 1992 harvest years. A second objective was to determine the TMEn of each of the six wheat samples in adult cockerels. The third objective was to examine the relationships between the productive performance of the broilers and the ME values and the chemical or physical characteristics of the six wheat samples.

#### **4.1.2. Treatments**

The six samples of Dean and Beaver from 1990, 1991 and 1992 were fed as part of nutritionally adequate broiler diets in the experiment.

The diets were formulated with the wheat samples included at a level of 700 g/kg, as detailed in section 2.2.

#### **4.1.3. Productive Performance And AMEn Protocol**

Ninety six male Ross-strain broiler chicks were used. They were fed a conventional broiler starter diet and kept in a littered floor pen until 7 days old (average weight 0.144 kg per bird). The birds were then randomly allocated, in pairs, to 48 cages. The cages measured 0.3m x 0.3m x 0.3m, with a floor mesh size of 1.5 cm x 1.5 cm, and were arranged in eight randomised blocks. The birds were given *ad libitum* access to feed and water from troughs, attached to the front of the cages, which measured 11 cm x 11 cm and 10 cm x 8 cm respectively. The birds were maintained at a controlled temperature (which started at 30°C and declined to 25°C over the experimental feeding period), with a 23.5 hour lighting regime. Each cage of birds within the block was randomly allocated one of the six experimental diets, to give eight cage replicates per diet. The experiment lasted for 14 days, during which time weight gains and feed intakes were measured. Feed intake was measured and excreta were collected over the last four days of the feeding period, and AMEn was determined.

#### 4.1.4. TMEn Protocol

The TMEn values of the diets were determined using a method modified from that described by McNab & Blair (1988). Twenty one adult layer-strain cockerels (average weight 3.2 kg per bird) were housed singly in cages, measuring 0.6m x 0.7m x 0.6m with a 2.5 cm x 2.5 cm floor mesh, at a constant house temperature of 16°C and with a 16 hour lighting regime. The experimental protocol is summarised in table 4.1. Birds were tube-fed either a milled wheat sample or a sucrose solution, and had *ad libitum* access to water from nipple drinkers. Three bird replicates for each wheat sample were used per experiment, and the protocol was conducted three times in order to give 9 replicates for each wheat sample. Three birds in each experiment were used as controls in order to calculate endogenous energy losses for the animals. The wheat samples and control dietary treatments were allocated to the cages randomly for each experiment.

The birds were tube-fed directly into the crop using a stainless steel tube (Sibbald, 1985). Excreta were collected on trays under each cage; those collections contaminated by regurgitated food were classed as missing values.

The collected excreta were dried in an oven at 60°C (following the recommendations of Blake & Potter, 1987). Excreta dry matter weight, gross energy and nitrogen was recorded in order to determine TME<sub>n</sub> value.

The cockerels were then maintained on a grower diet *ad libitum* for nine days after the end of the experimental period, during which time their body weight recovery was recorded.

**TABLE 4.1 TME DETERMINATION PROTOCOL**

<b>Time (hours)</b>	<b>Action</b>
0	All food removed from birds.
24	All birds tube-fed 50 ml 70% (w/v) sucrose solution.
48	Experimental birds tube-fed 50g milled pure wheat sample. Control birds tube-fed 50 ml 70% (w/v) sucrose solution.
	Excreta collection started.
72	All birds tube-fed 50 ml water.
96	Excreta collected, dried and weighed. <i>Ad libitum</i> feeding resumed.



#### **4.1.5. Statistical Analysis**

The results of the productive performance and AMEn trial were analysed as a two-factor, randomised block analysis of variance. Wheat variety and harvest year were used as treatment factors, and the experimental data was blocked by cage block. A factorial, randomised block analysis of variance design was used for the TMEn data. Again, the treatment factors were wheat variety and harvest year and replication over time was used as a blocking factor. A correlation matrix to investigate the relationship between broiler growth and feeding efficiency (FCR) and ME measurements was constructed.

#### **4.1.6. Results**

The productive performance, AMEn and TMEn data are shown in table 4.2 below, and in graphs 4.1, 4.2 and 4.3. There were four missing values recorded for the broiler growth trial due to death of birds, and five missing values recorded for the TME results, due to regurgitated feed in the excreta.

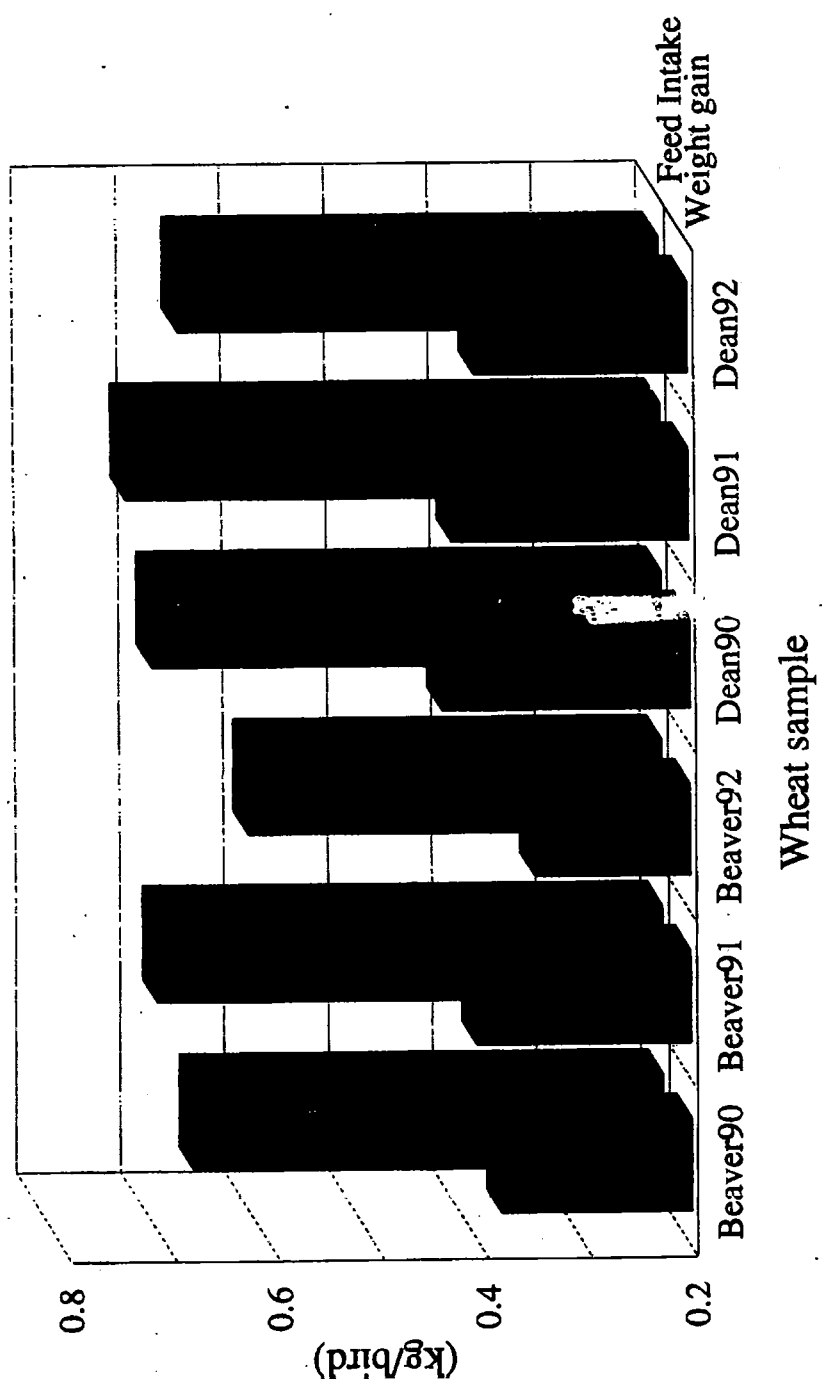
**TABLE 4.2. PERFORMANCE AND NUTRITIONAL VALUES FOR SAMPLES OF DEAN AND BEAVER**

**HARVESTED IN 1990, 1991 AND 1992**

Variety	Year	Weight gain (kg/bird)	Feed intake (kg/bird)	FCR (kg/kg)	AMEn (MJ/kg DM)	TMEn (MJ/kg DM)
Beaver	1990	0.382	0.650	1.702	13.59	14.67
	1991	0.405	0.685	1.691	13.51	14.72
	1992	0.349	0.597	1.710	13.12	14.37
Mean		0.379	0.644	1.701	13.40	14.59
Dean	1990	0.437	0.689	1.577	13.67	14.91
	1991	0.427	0.713	1.670	13.30	14.58
	1992	0.404	0.662	1.639	13.58	14.57
Mean		0.423	0.688	1.629	13.52	14.69
SEM Variety		0.0078***	0.0113**	0.0117***	0.060	0.035
SEM Year		0.0096*	0.0138**	0.0143	0.073	0.043***
SEM Variety x year		0.014	0.0195	0.0203*	0.104	0.061*
Residual DF		31	31	31	31	35

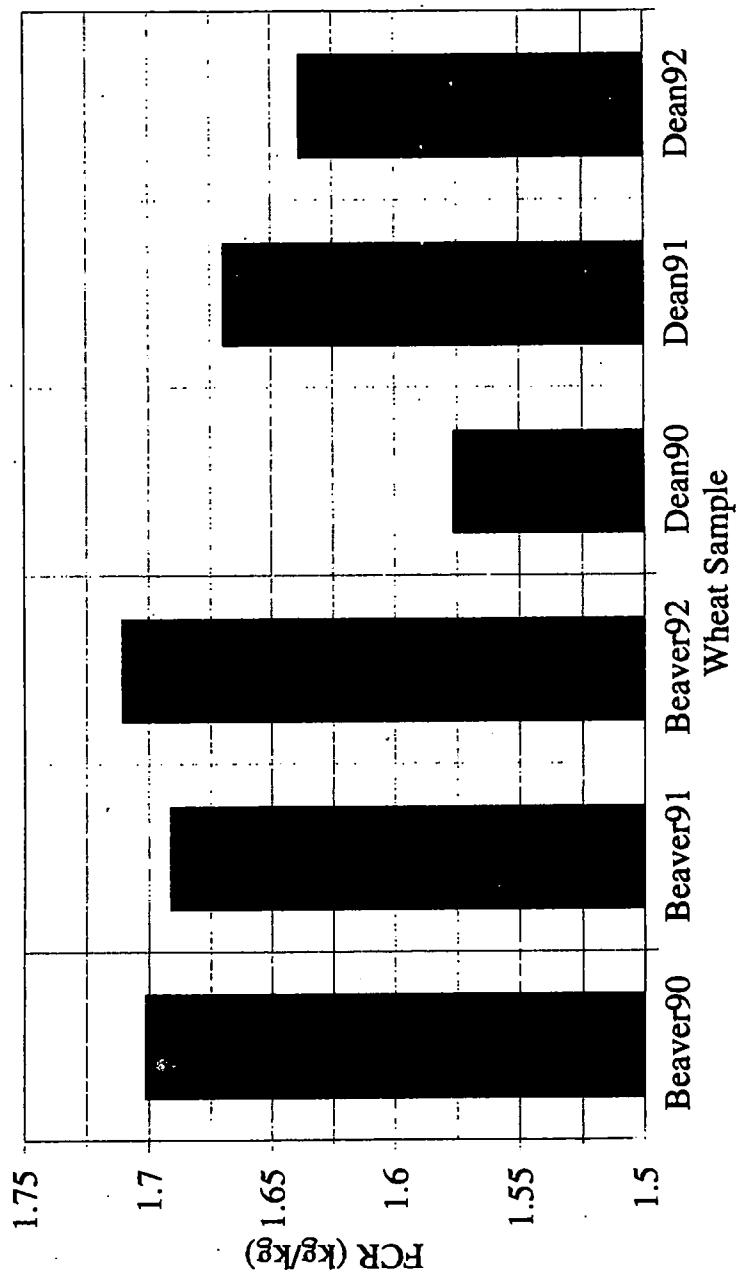
\*p<0.05; \*\*p<0.01; \*\*\*p<0.001

**GRAPH 4.1. WEIGHT GAIN AND FEED INTAKE OF BROILERS FED DEAN OR BEAVER WHEAT SAMPLES HARVESTED IN 1990, 1991 OR 1992**

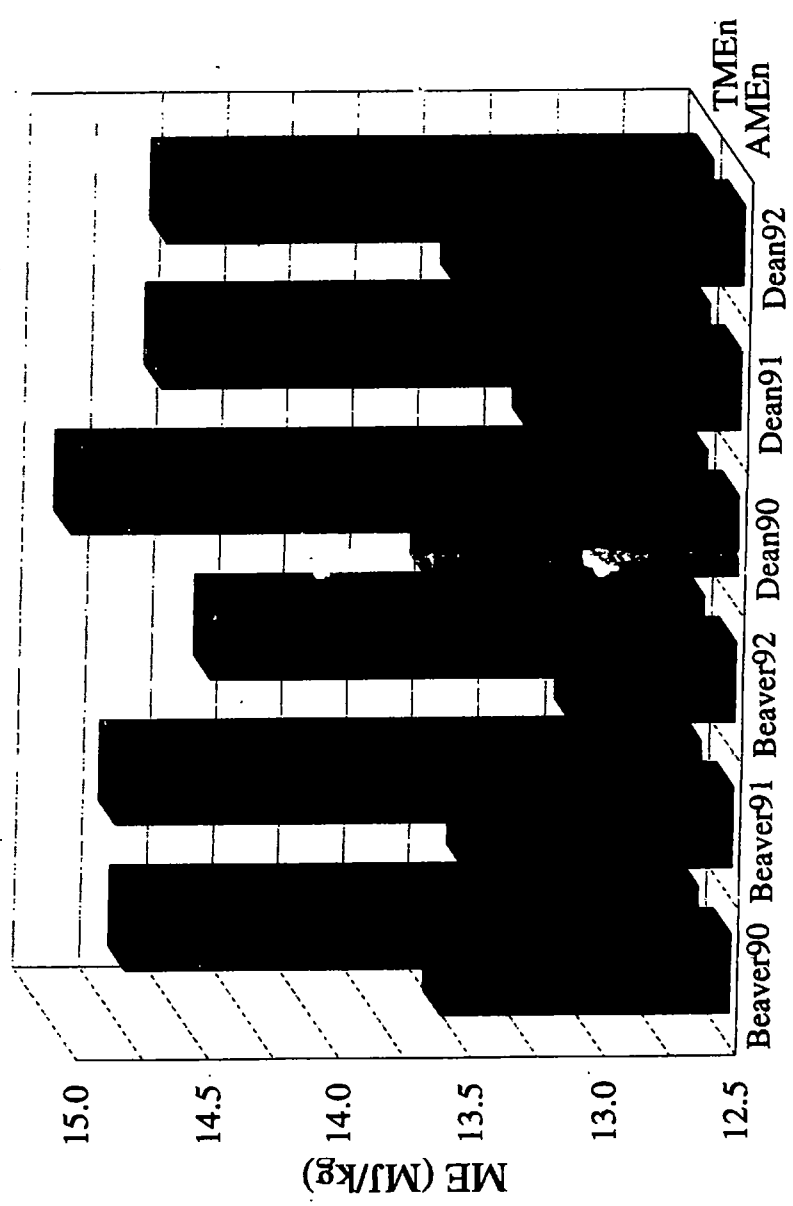


**GRAPH 4.2. FEED CONVERSION RATIO OF BROILERS FED DEAN OR BEAVER WHEAT SAMPLES**

**HARVESTED IN 1990, 1991 OR 1992**



**GRAPH 4.3. APPARENT AND TRUE METABOLISABLE ENERGY VALUES FOR BROILERS FED DEAN OR BEAVER WHEAT SAMPLES HARVESTED IN 1990, 1991 OR 1992**



There were significant differences in weight gain ( $p<0.001$ ), feed intake ( $p<0.01$ ) and FCR ( $p<0.001$ ) between the two wheat varieties Dean and Beaver. Significant differences in weight gain ( $p<0.05$ ) and feed intake ( $p<0.001$ ) existed between the three harvest years, and significant differences ( $p<0.05$ ) for FCR were found between the variety means within harvest years (variety x year interactions).

The diets formulated with the variety Dean resulted in the highest broiler weight gains and feed intakes (12% and 7% greater than Beaver respectively) and improved FCR by 4%. These differences were evident over all three harvest years. Wheat samples harvested in 1992 showed the poorest broiler productive performance, with 9% lower weight gains and 8% lower feed intakes than the combined means of 1990 and 1991 wheat samples from both varieties.

The broiler productive performance was measured as weight gain, feed intake and FCR over the whole 14 day growing period. The grand means of broiler performance were 0.401 kg/bird weight gain, 0.666 kg/bird feed intake and 1.67 kg/kg FCR. The AME values determined for Dean and Beaver from 1990, 1991 and 1992 harvests had a grand mean of 13.46 MJ/kg when corrected to zero nitrogen balance and

expressed on a dry matter basis (AMEn). The AMEn means of the six experimental wheat diets were not significantly different, and there were no treatment interactions.

The TME values determined for the wheat varieties Dean and Beaver from 1990, 1991 and 1992 harvests had a grand mean of 14.64 MJ/kg, when corrected to zero nitrogen balance and expressed on a dry matter basis (TMEn). There were no significant differences ( $p>0.05$ ) in TMEn between the two wheat varieties, however there was a significant difference between the harvest years ( $p<0.001$ ), and a variety x year interaction ( $p<0.05$ ). This was due to the high value for Dean 1990 and the low value for Beaver 1992 samples. When the TMEn data from the other four wheat samples was analysed separately, no significant differences were observed (grand mean 14.65 MJ/kg; SEM=0.065, residual DF=21;  $p>0.05$ ).

Correlation matrices were constructed to identify whether results for the experiments conducted in section 3 were related to the growth of the broilers in this experiment. The regression coefficients ( $r$  values) are shown in tables 4.3, 4.4, and 4.5 below. There was a strong

TABLE 4.3. CORRELATION MATRIX FOR BROILER GROWTH AND METABOLISABLE ENERGY

MEASUREMENTS

	Weight gain	Feed intake	FCR	AME <sub>n</sub> DM	TME <sub>n</sub> DM
Weight gain	1.00				
Feed intake	0.936**	1.00			
FCR	-0.814*	-0.487	1.00		
AME <sub>n</sub> DM	0.576	0.454	-0.607	1.00	
TME <sub>n</sub> DM	0.760	0.644	-0.695	0.838*	1.00

\* p&lt;0.05; \*\* p&lt;0.01

DF = 4



correlation between weight gain and feed intake ( $p < 0.01$ ). The efficiency of feed utilisation, measured as FCR, was also related ( $p < 0.05$ ) to weight gain, but not to feed intake. There was a correlation ( $r = 0.838$ ,  $p < 0.05$ ) between the AMEn and TMEn values, even though those measurements were not related to the growth and performance of the birds.

The second correlation matrix was constructed to determine any relationships between the chemical or quality characteristics of the wheat and the performance of the broilers. The only relationships linking broiler growth and the chemical analysis of the samples was between the dry matter content of the wheat and weight gain ( $r = -0.949$ ,  $p < 0.01$ ), feed intake ( $r = -0.910$ ,  $p < 0.05$ ) and FCR ( $r = 0.841$ ,  $p < 0.05$ ). There were also significant relationships between broiler weight gain and specific gravity ( $r = -0.879$ ;  $p < 0.05$ ), and FCR and HFN ( $r = -0.838$ ;  $p < 0.05$ ).

**TABLE 4.4. CORRELATION MATRIX FOR BROILER GROWTH AND CHEMICAL OR QUALITY**

**ANALYSIS**

	Dry matter	Crude Fibre	Oil	Crude Protein	Specific Gravity	Relative Hardness	HFN	Particle size
Weight gain	-0.949**	-0.719	-0.373	-0.310	0.879*	0.698	0.733	-0.630
Feed intake	-0.910*	-0.694	-0.634	-0.597	0.726	0.798	0.604	-0.410
FCR	0.841*	0.627	-0.053	-0.056	-0.809	-0.446	-0.838*	0.766

\* p<0.05; \*\* p<0.01

DF = 4

The final correlation matrix (table 4.5) was constructed to determine any relationships between broiler performance and the nature of the carbohydrate in each of the wheat samples. Broiler weight gain and feed intake was significantly related to the amount of free glucose present in the wheat sample ( $r=-0.981$  and  $r=-0.944$  respectively,  $p<0.001$ ), but not to the total starch. There were no significant relationships between bird growth and total starch.

**TABLE 4.5. CORRELATION MATRIX FOR BROILER GROWTH AND CARBOHYDRATE ANALYSIS**

	Free	Total	Total	Insoluble	Soluble	AM:AP
	Glucose	Starch	NSP	NSP	NSP	ratio
Weight gain	-0.981***	0.558	-0.100	0.468	-0.401	-0.521
Feed intake	-0.944***	0.759	-0.173	0.685	-0.594	-0.302
FCR	0.729	-0.291	0.107	0.100	0.116	0.750

\* p<0.05

DF = 4

#### 4.1.7. Discussion

The results of the experiment indicated that the hard variety Dean, had a greater nutritive value for growing broiler chickens than the soft variety, Beaver. Differences in broiler weight gain between hard and soft UK wheat varieties had been reported by Rose *et al* (1992), who found a 9% difference between the means of the wheat samples, when grouped by endosperm texture characteristics (these varieties included Dean and Beaver). Further research following on from their studies was conducted by Bonnet (1994), who found a 10% increase in weight gain ( $p>0.05$ ) and feed conversion efficiency (the inverse of FCR) ( $p<0.05$ ) in broiler chicks fed the variety Riband compared to those fed Haven, although Riband was the marginally softer variety. These results suggested the reverse relationship to that put forward by Rose *et al.* (1992). No measurements of starch content, quality or availability for digestion were made during their studies, so any relationship between these parameters and broiler growth could not be evaluated.

Similar growth and feeding efficiency differences were recorded during the experiments using 33 Canadian wheat samples conducted by March and Biely (1973), who found that feed wheat varieties could be

ranked according to broiler productive performance. One variety, Pitic, was identified as the nutritionally poorest sample, with significantly poorer weight gains and feed conversion efficiency when compared to the mean of all the other samples studied.

According to the small amount of published work concerning the effect of climate and harvest year on the nutritional value of wheat for broilers, deterioration in the nutritive value of the wheats over the three harvests was probably attributable to a decline in weather conditions. Wiseman (1990) outlined how poor weather conditions during final grain filling stages and harvest often lead to reduction in the nutritional quality of feed wheat, which is often reflected in the production efficiency of broiler chicks. In their wheat composition and growth experiments, Rose *et al.* (1992) noted that weather differences due to growing season altered the productive response of broiler chicks and the HFN (which is possibly related to grain filling (Wiseman, 1990)) of the wheat variety samples.

The AMEn values reported on a dry matter basis were in a range similar to values reported by Mollah *et al.* (1983) and Rose *et al.* (1992). Rose *et al.* (1992) recorded lower AMEn values for Dean and Beaver than obtained in this trial, but reported significant varietal differences. No

differences in determined AME value, however, were found by Rogel *et al.* (1987) , Wiseman (1990) or Salah Uddin *et al.* (1996). The variation in significance and magnitude of AMEn values for Dean and Beaver between this experiment and those previously conducted by Rose *et al.* (1992) supported the recently expressed argument that the ME values for samples of feed wheat are highly variable and difficult to quantify consistently (Mollah *et al.*, 1983; Nichol *et al.*, 1993; Wiseman *et al.*, 1994; McNab, 1990, 1995 and Rose & Bedford 1995). It has also been observed that both AME and TME do not relate to broiler performance when compared directly from data within the same growth trial (Rose & Bedford, 1995).

The TME values were comparable to the ranges recorded by McNab (1991) for 17 UK wheat varieties, and Sibbald (1977) for 39 Canadian wheat samples. The absence of significant differences in TME values between the wheat varieties agreed with the reported TME results reported by Longstaff and McNab (1986). Their work included an investigation into digestibility and ME values of six wheat varieties from different growing sites. They found that the TMEn values, expressed on a dry matter basis, for the six varieties showed no significant variation. There were, however, differences in carbohydrate content due to wheat

variety and growing site that were not reflected in TMEn value. No data regarding the performance of broilers fed these varieties was included in the report, so the effects of carbohydrate content on nutritional value could not be evaluated.

In 1991, McNab examined possible factors affecting the nutritive value of wheat (expressed as TME value), and the influence of wheat variety or environmental conditions (growing year and site) on such factors. He found no differences in TMEn, when expressed on a dry matter basis, between the wheat samples. He could not identify any causal factor that was directly related to differences between wheat varieties and nutritional content. More interestingly, McNab (1991) also could not detect a significant difference in the TME value for the variety Slejpner, which had been widely reported from commercial sources to result in poor broiler feeding and growth efficiency in comparison with other varieties.

Recent work presented by McNab (1996) showed that TME values for the same wheat samples varied between experiments and laboratories. He concluded from this that variation in determined ME values for wheat does not explain nutritional differences. It appeared that TME values



differed between wheat samples for unknown reasons and by unknown mechanisms, which may or may not have been related to the digestion characteristics or other nutrient quality of the sample. From this work it was hypothesised that detection of significant differences in the feeding quality expressed as TME between groups of wheat samples could be inconsistent and unreliable. Certainly the use of TME values, derived from adult birds, may not seem directly applicable as a nutritional value required for use in very young, fast growing broiler chicks. The main reasons for this have been identified as the large differences in the digestive physiology, endogenous enzyme production, metabolism and partitioning of dietary energy for growth and maintenance between these two groups of chickens (Bedford, 1995). In the case of this experiment, however, it would appear that AMEn and TMEn values may be related to each other, as a significant correlation was found between these two values (table 4.3). This was to be expected as the TMEn method was derived from and is therefore strongly related to the AMEn method (Sibbald, 1985).

The lack of significant differences between any of the TME values due to wheat variety and year in our experiment indicated that TME could

not be used as a method of determining the nutritive value of the wheat varieties Dean and Beaver for growing broiler chickens.

The strong relationship between weight gain and feed intake showed that increased feed intake was largely responsible for the increases in growth between broilers fed the six diets. Since, in addition to this, FCR was related to weight gain, but not feed intake, the results suggest that nutrient availability to the animal was not dictated purely by volume of feed, but could be linked to digestibility of the diet.

The absence of a relationship between the determined AMEn or TMEn and the weight gain, feed intake and FCR of the broiler chicks indicated that ME was not a method that could be applied to indicate the true nutritive value of these wheat samples in complete poultry diets. There may be a number of reasons why ME does not account for variation in feed quality. Methodology for the determination of ME assumes that energy loss, in the form of body heat and respiratory losses, remains constant between individual birds (McDonald *et al.* 1981). No account is made for the proportion of hind-gut fermentation occurring and the amount of dietary energy lost to support the caecal microflora colonies (Rose & Bedford, 1995), or for differences in the metabolic

efficiencies of the fermentation products. The host bird will utilise volatile fatty acids evolved from fermentation in different ways depending upon their molecular complexities (McDonald *et al.* 1981).

The majority of the research published regarding nutritive value of feed has been concerned with attempts to find a laboratory method that can be used to predict the AME of broiler feed (Mollah, 1983; Carré, 1990; Campbell *et al.*, 1985; Wiseman, 1990; Wiseman *et al.*, 1993). The relationship between cereal composition and broiler growth has yet to be quantified.

Values for wheat dry matter did not show sufficient variation between samples to be used as an estimate of nutritional value despite the relationship found linking broiler growth and wheat dry matter content. Those wheats with the lowest dry matter did however give the highest weight gains and feed intakes, and the most efficient conversion of feed to weight gain. Protein content was the most statistically different chemical component between the six wheat samples. However, these variations were not linked to broiler growth differences, suggesting that the levels of protein in the diet were all adequate for broiler growth requirements, and additional protein supply gave no further improvement

in performance. These findings support those of Rose *et al.* (1992) who also investigated chemical composition during their work on the feeding quality of six UK wheat varieties (including Dean and Beaver), and found no significant relationship between wheat composition and broiler performance.

The significant relationships between broiler weight gain and specific gravity were possibly indicative of the amount of available starch in proportion to bran, as larger, better filled grains (such as Dean) may be considered to contain more starch in relation to bran than small, shrivelled grains. If this was the case, then the diets formulated with the better filled grains could contain proportionally more starch in a readily digestible form (Wiseman, 1990), which would be available to the birds for growth when broken down to glucose by digestive processes. As there was no correlation between specific gravity and AMEn value, it had to be assumed that there were other factors (see section 1.5.3. (iii)) which masked any differences in digestibility that should have been correspondingly observed in AMEn (Wiseman & Inborr, 1990; Rose *et al.*, 1992; Wiseman, 1992; McNab, 1996; Rose & Bedford, 1995). It may also further support the theory that AMEn values are too variable to detect such differences (Annison & Johnson, 1989; Annison, 1993). The

significant relationship between HFN and FCR signified that wheats with higher HFN were more efficiently utilised by broilers when fed as complete diets. This effect could possibly be a result of the higher HFN wheats solubilising more quickly within the gut of the broiler, as HFN may be considered as an indicator of level of starch gelatinisation (Hagberg 1960, 1961). The liberated soluble starch would therefore have been more readily available for enzymic attack, and therefore more efficiently digested and assimilated by the bird.

The differences in free glucose between the wheat samples, although highly significant, were far too small ( $< 1\%$ ) to account for the major differences in bird weight gain ( $>10\%$ ). This is despite the significant relationship found between wheat free glucose and both broiler weight gain and feed intake. The response to free glucose suggested that this could have been related to the similar response observed for Hagberg falling number and broiler FCR, in that the amount of free glucose could indicate a nutritional quality difference (improved HFN), even though it was not responsible for that difference (Best & Muller, 1991; Gruppen, 1996). The lack of any relationships between bird growth and total starch were unexpected, since there was a relationship noted between weight

gain and specific gravity, the latter being considered a measure of the 'grain fill', i.e. starch packed into the endosperm.

No relationship was found between the AM:AP ratio and any of the broiler growth characteristics, even though the chemical composition of the starch varied significantly between the two varieties. The higher levels of amylose present in Beaver therefore could not have been inhibiting digestion within the gut (Behall *et al.*, 1989; Åman & Graham, 1990).

There were no significant differences in NSP content between the wheat varieties, and correspondingly no correlation between any NSP fraction and the growth of the broilers. Choct & Annison (1992) and Annison (1993) had reported a close correlation between NSP and reduced broiler weight gain and feed utilisation for broiler chicks fed Australian wheat. However Wiseman & Inborr (1990) had previously commented that only cereals with high levels of NSP appeared to influence broiler growth. Annison (1990) and Nichol *et al.* (1993) both reported that NSP was not related to the apparent digestibility of wheat starch. However the effects on broiler weight gain and FCR were not reported, therefore it was only possible to speculate that there was no

relationship between NSP and bird performance for their experiments. Soluble NSP has been identified with reduced AME values (Annison, 1993), however when the data from our trial was examined, no significant relationship between the soluble NSP fraction and determined AME<sub>N</sub> was found ( $r=-0.730$ , 4DF,  $p>0.05$ ). This was thought to be due to the lack of significant variety differences for these parameters, as well as the possible variability of AME values for wheat diets (Rogel *et al.*, 1987; Annison & Johnson, 1989; Wiseman & Inborr, 1990; Annison, 1993) and the relatively low levels of NSP in UK wheat ( McNab, 1993).

## **4.2 NUTRITIONAL COMPARISON AND DIGESTA VISCOSITY OF DEAN AND BEAVER OVER WHOLE BROILER GROWING PERIOD**

### **4.2.1. Objectives**

The main objective of this experiment was to determine whether differences in broiler growth or performance, when fed Dean or Beaver from different harvest years, were consistent over three different ages that encompassed a typical commercial growing period of broilers. The second objective was to investigate if there were digesta viscosity differences due to variety and whether this was related to productive performance.

### **4.2.2. Treatments**

Dean and Beaver from 1991 and 1992 only were used in this experiment, due to diminished availability of samples of the varieties harvested in 1990. The diets were formulated as described in section 2.2, using the wheat samples at 700 g/kg wheat inclusion.



#### 4.2.3. Productive Performance And Digesta Viscosity Protocol

Two hundred and eighty eight male broiler chicks were reared on conventional broiler starter diets and kept in a litter-floor pen until 7 days old, with an average body weight of 0.154 kg per bird. Ninety six of these birds were housed in pairs in 48 cages, measuring 0.3m x 0.3m x 0.3m, with a floor mesh size of 1.5 cm x 1.5 cm. Diets were allocated to cages according to a randomised block design. The cages were arranged in four tiers, with three diet replicates per tier. The birds had *ad libitum* access to feed and water, supplied from vessels measuring 11 cm x 11 cm and 10 cm x 8 cm respectively, attached to the outside of the cages. The house was maintained at a controlled ambient temperature, starting at 30°C and declining to 25°C over the 14 day experimental feeding period, with a 23.5 hour lighting regime. The remaining 192 birds were maintained in the litter-floor pen and fed an *ad libitum* diet identical to the experimental diet except that the wheat used was commercial feed wheat obtained from the Harper Adams feed mill. During the 14 day experimental feeding period, weight gains and feed intakes per cage were measured. Faeces were collected on the last four days of the experiment and AMEn values were calculated.

The next 96 birds were then randomly selected from the original flock, paired and transferred to the same cages and the experiment repeated to give data for birds aged 21-35 days old (0.710 kg average weight per bird at 21 days old). The final 96 birds used in the 35-49 day old experiment (1.510 kg average weight per bird at 35 days old), and were treated in the same way as the previous two groups of birds. However, because of their size and the necessity of housing birds in pairs, they were placed in larger cages, measuring 0.4m x 0.4m x 0.4m, with a 2.5 cm x 2.5 cm floor mesh. The birds used in the 21-35 day old and 35-49 day old experiments were housed at a constant temperature of 25 °C.

The digesta viscosity of the birds was measured, according to a method devised by Finnfeeds International Ltd., on the last day of the 14 day feeding period of each of the three experiments. Each bird was killed by cervical dislocation and dissected, and the portion of the small intestine from the end of the duodenal loop to the Merckels Diverticulum (the vestigial yolk sack) was removed. The contents of the gut section were expelled into a cup, thoroughly mixed and transferred into two eppendorf 2 ml micro-centrifuge tubes, which were immediately spun in a micro-centrifuge at 3000 rpm for three minutes in order to isolate the

liquid portion of the digesta. The viscosity of a 0.5 ml aliquot taken from the liquid portion in each of the tubes was then measured using a Brookfield digital viscometer, to give two digesta viscosity measurements per bird replicate.

#### **4.2.4. Statistical Analysis**

Each age group of broilers gave feeding efficiency and growth results characteristic of the maturity of the birds. This data could not be directly compared due to differences in the variance between the measurements within the different age groups (as confirmed by Bartlett's test of Homogeneity (Cochran & Cox 1957)). This necessitated the transformation of all the data into values that were directly comparable, i.e. of similar SEM (Mead & Curnow, 1983), in order to meet the assumptions of the analysis of variance procedure. The weight gain, feed intake and FCR results were transformed by expressing each data point as a percentage of the published Cobb 1995 average values for that parameter at that age (table 4.6). Each experimental data point was divided by its corresponding Cobb value (for FCR the Cobb value was divided by the experimental data, due to the inverse nature of the relationship between FCR and broiler growth), and then multiplied by

100. It was not necessary to transform the AMEn and foregut digesta viscosity data because their SEMs were not changed by the age of the broiler. The results of the whole growing period were statistically analysed as an analysis of variance, with bird age, wheat variety and harvest year used as treatments, and blocked for diet replicates.

**TABLE 4.6. COBB AVERAGE GROWTH PERFORMANCE OF BROILERS USED FOR TRANSFORMING THE EXPERIMENTAL DATA**

Broiler age (days old)	Weight gain (kg/bird)	Feed Intake (kg/bird)	FCR (kg/kg)
7 - 21	0.640	0.952	1.36
21 - 35	0.929	1.742	1.63
35 - 49	0.995	2.383	1.91

(Cobb 500 Broiler Performance, 1995.)

#### **4.2.5. Results**

The results are displayed in table 4.7 and graph 4.4 below. There were significant varietal differences in the productive performance of the broiler chickens aged from 7-49 days old. Broilers fed Dean, on average, had 9% higher weight gains ( $p < 0.001$ ) and 5% higher

**TABLE 4.7. PRODUCTIVE PERFORMANCE AND DIGESTA**  
**VISCOSITY FOR BROILERS FED DEAN OR BEAVER WHEAT**  
**SAMPLES HARVESTED IN 1991 OR 1992 OVER A TYPICAL**  
**COMMERCIAL GROWING PERIOD**

Variety / Year		Weight gain (% Cobb)	Food Intake (% Cobb)	FCR (% Cobb)	AMEn DM (MJ/kg)	Viscosity (cP)
7-21 days old						
Beaver	1991	90.85	94.81	87.54	12.50	10.19
	1992	83.20	87.33	87.05	13.16	10.70
	Mean	87.02	91.07	87.29	12.83	10.45
Dean	1991	96.69	97.77	90.42	12.04	7.59
	1992	86.76	88.81	89.24	13.29	9.64
	Mean	91.73	93.29	89.83	12.66	8.61
21-35 days old						
Beaver	1991	62.12	70.34	75.92	11.02	9.07
	1992	58.94	65.17	77.65	13.58	5.77
	Mean	60.53	67.76	76.78	13.75	7.42
Dean	1991	63.38	69.28	79.14	13.44	6.58
	1992	72.85	72.65	86.89	13.57	6.10
	Mean	68.12	70.96	83.02	13.50	6.34
35-49 days old						
Beaver	1991	75.40	73.56	81.71	13.58	8.84
	1992	69.70	69.22	79.71	13.27	8.42
	Mean	72.55	71.39	80.71	13.43	8.63
Dean	1991	80.86	77.80	82.57	13.59	7.96
	1992	80.43	75.16	86.53	13.45	8.41
	Mean	80.64	76.48	84.55	13.52	8.18
SEM:						
age		1.529***	0.913***	1.338**	0.069***	0.369**
variety		1.249***	0.745**	1.092**	0.056	0.301*
year		1.249	0.745***	1.092	0.056*	0.301
age x variety		2.163	1.291	1.892	0.097	0.522
age x year		2.163	1.291*	1.892	0.097***	0.522*
variety x year		1.766	1.054	1.545	0.079*	0.426
age x var. x year		3.059	1.825	2.675	0.137	0.738
Residual DF		108	108	108	118	118

\*p<0.05; \*\*p<0.01; \*\*\*p<0.001

feed intakes ( $p < 0.01$ ) than those fed Beaver. Feed conversion ratio for Dean was 5% lower than Beaver ( $p < 0.01$ ) indicating improved feed efficiency for birds fed the former diets. The digesta viscosity of birds fed Dean was 11% lower ( $p < 0.05$ ) than those fed Beaver-based diets over all three age groups. AMEn was not affected by wheat variety.

The age of the broiler had a significant impact on its weight gain, feed intake ( $p < 0.001$ ) and FCR ( $p < 0.01$ ), and there were no age x variety interactions. The youngest age group (7-21 day old birds) ate and grew proportionally more than the older birds and were more efficient at converting Dean diets into tissue growth.

Differences between harvest year were seen in feed intake ( $p < 0.001$ ) and AMEn ( $p < 0.05$ ). The 1991 samples, on average, gave higher feed intakes than those harvested in 1992. There was a significant interaction between year means within the age groups ( $p < 0.05$ ) for feed intake values, which was due to the large significant difference between harvest year means in feed intake values altering in magnitude over the three age groups. The significant variation in AMEn between harvest years was probably due to the decrease in value for samples of Dean from 1991 in contrast to the means for the other three wheat samples (Beaver

1991 and Dean and Beaver 1992). AMEn values were also affected by the age of the bird ( $p < 0.001$ ), in that the values increased over the 7-35 day old broiler growing period. There were significant variations in AMEn value between harvest year means within age groups and between variety means within harvest years (age x year interaction,  $p < 0.001$ ; variety x year,  $p < 0.05$ ). However there were no overall differences ( $p > 0.05$ ) between the varieties Dean and Beaver.

The birds fed Dean-based diets had lower digesta viscosities over all three ages when compared to those fed Beaver. Although there was no significant interaction ( $p > 0.05$ ) between bird age and variety for viscosity, the magnitude of the numeric differences between the variety means declined with bird age. The youngest birds showed the largest differences (21%) between varieties in terms of digesta viscosity, whereas the 21-35 day old broilers showed a 17% difference, and the oldest bird group showed a 6% difference. An interaction between harvest year means within age groups was observed in viscosity measurements, suggesting that the environmental effects on wheat composition caused a response in foregut viscosity that was determined by the age of the bird.

Bedford and Classen (1992) examined wheat and rye based diets with a wide range of viscosities in chicken foreguts, and, from their experiments, found a logarithmic relationship between the viscosity and FCR of broilers at 21 days old. The same regression analysis was therefore performed on the data from this experiment. There was no significant relationship between the logarithm of the viscosity and the FCR in this case, either for the youngest broilers ( $r=0.194$  with 46 DF for 7-21 days old birds) or over all three groups ( $r=0.084$  with 137 DF for 7-49 days old birds).



#### 4.2.6. Discussion

The results of this experiment indicated that productive performance and digesta viscosity differences due to wheat variety were present over the whole growing period of the broiler chickens (7-49 days). The experimental results for broiler productive performance were consistently lower than the published Cobb values. The effects of harvest year on the results of this experiment were different to those in the previous feeding experiments, because wheat samples from only 1991 and 1992 were used. These two harvest years gave increasingly poorer grain filling, and were both determined as nutritionally poorer than the 1990 wheat samples in the previous animal growth experiment. The differences in growth and feeding efficiency of the birds were much less between the 1991 and 1992 samples.

There were no age x variety interactions for weight gain, feed intake or FCR of the broiler chickens. This demonstrated that the nutritional differences between the two wheat varieties, Dean and Beaver, were not dependant on bird age, with Dean giving better broiler performance compared to Beaver in each age group. This is of significant commercial importance as older broilers account for the majority of feed costs in

broiler production, and therefore any improvements in the efficiency of feed utilisation (FCR) represents a saving for broiler growers.

There is very little published information regarding the effect of cereal nutritional quality on broiler productive performance at any age, let alone at different bird ages. Most of the research has concentrated upon the effects of gut maturation on digestive processes. Pettersson & Åman (1989) found that improvements seen in broiler productive performance due to the addition of exogenous enzyme to the diet lessened as the birds grew older. The percentage improvement in performance due to enzyme was halved in broilers aged 27 days compared with the performance of 15 day old birds. Bedford (1995) commented that, although growth rate has yet to be shown to be directly linked to gut maturity, it would be logical to expect the anti-nutritive effects of viscous grain such as barley and wheat to decline as the animal's gut developed. The mature gut appears to be more efficient at breaking down the viscous NSP content of its food, which is manifested in improved nutrient availability and uptake. Bedford added that it could take several weeks of growth before pancreatic enzyme production was at such a level whereby growth rate reached a maximum and was no longer constrained by poor gut efficiency. These theories support the occurrence of a decline in the

differences in digesta viscosity between the two wheat varieties seen during this experiment over bird age. It was speculated from the experimental results that, as the bird matured it became more adept at breaking down the viscous elements in its diet, resulting in a reduction in the significant differences observed for viscosity between the wheat varieties over time. This hypothesis was suggested by the findings of Rogel *et al.* (1987) who measured the *in vivo* digestibility of Australian wheat samples, and reported that digestibility of wheat starch improved with broiler age.

The lack of any significant logarithmic relationship between viscosity and FCR was probably as a consequence of the limited range in viscosity observed in this experiment. In their investigations, in which this relationship was found to be significant, Bedford and Classen (1992) had a far larger range of viscosity and broiler performance data, their experiments using wheat and rye diets producing viscosity readings in the range 5 to 300 cP.

## **5. INVESTIGATION INTO STARCH DIGESTIBILITY**

### **5.1. IN VIVO DIGESTIBILITY**

#### **5.1.1. Objectives**

The objective of the experiment was to investigate whether differences in digestibility between the varieties Dean and Beaver could be detected in samples of feed taken from the gut of chickens during the natural digestive process. The value of these experimental results as an indicator of digestive efficiency, and for identifying those features that could be adapted for use as possible methods of assessing the true nutritional quality of the wheat samples were examined.

#### **5.1.2. Treatments**

The small amounts of Dean and Beaver remaining from the 1990 harvest after the feeding trial (section 4.1) were used in this experiment. They were fed at 700 g/kg inclusion in complete broiler rations formulated to the same specification as in the previous broiler growth trials (section 2.2). Only two samples, one of each wheat variety,

were used in order to maximise bird replicates during the trial, whilst still keeping the amount of sample collection, preparation and analysis to a manageable level. The variety samples from 1990 had previously shown the largest differences in broiler growth and performance, and so were expected to give the largest, and most easily detectable differences in *in vivo* digestion results. Chromium oxide ( $\text{Cr}_2\text{O}_3$ ) was added to the finished meal-form diet as a digestibility marker at a level of 1.5 g/kg.

### **5.1.3. Experimental Protocol**

Twenty-four, individually caged, male broiler chickens, aged 14 days old, were assigned to one of the two diets, giving twelve bird replicates for each diet. The birds were fed according to a 12 hour restriction pattern, whereby they had *ad libitum* access to feed from troughs in front of the cages for 12 hours per day, followed by 12 hours of fasting. The experimental feeding period lasted for seven days.

The birds were housed in cages arranged in four rows; each cage was allocated one of the experimental diets according to a randomised block design. The cages used in this experiment were those previously used in the TME determinations (section 4.1.4). All birds were fed from

troughs (measuring 11 cm x 11 cm and attached to the outside of the cages) according to a 12 hour restricted feeding pattern. Each row had a different feeding regime; row 1 was fed between 7 a.m. and 7 p.m., row 2 between 8 a.m. and 8 p.m., row 3, 9 a.m. to 9 p.m., and row 4, 10 a.m. to 10 p.m.. This arrangement was to ensure that, when killed and dissected, all the birds would have guts full of feed, and all would have been feeding for approximately the same length of time (2 hours), despite the time differential between killing the first and last bird. A 15 hour lighting pattern (7 a.m. to 10 p.m.) was used to fit in with the feeding regime, and the broilers had *ad libitum* access to water from nipple drinkers at all times.

On the final day of feeding, each bird was killed and immediately dissected 2 hours after feed had been reintroduced to its particular row. The intestines were removed and cut into two sections, A and B as described in table 5.1, and the contents removed. A sample of fresh faeces was also taken. The samples of digesta or faeces were labelled, freeze-dried, ground in a pestle and mortar and analysed for chromium oxide. The samples were also assayed for free glucose and total starch content in order to determine differences between the wheat varieties in the levels of available glucose or disappearance of starch in the gut.

**TABLE 5.1. GUT SECTIONS**

<b><u>Sample code</u></b>	<b><u>Gut section</u></b>
A	Distal duodenal loop to Merckels diverticulum
B	Merckels diverticulum to Caecal junction
C	Fresh faeces

#### **5.1.4. Laboratory Analysis**

##### **(i) Chromium oxide**

The concentration of chromium oxide in each freeze-dried digesta or faecal sample was determined by atomic absorption using Thermo-Electron Atomic absorption apparatus. The samples were prepared according to a method devised by Parker (1990), where initially 1.0g of freeze-dried faeces were ashed for 12 hours at 550°C. A Kjeltec heating

block, drilled to a depth of 10 cm and with capacity for 20 digestion tubes, was then pre-heated to 150°C. The ashed digesta samples were transferred into Kjeltex digestion tubes, 6 ml of phosphoric acid-manganese mixture (30 ml of 10% (w/v)  $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$  solution mixed with 11 ml of 85% phosphoric acid) added, and the tube swirled to disperse the ash.

The tubes were then placed in the heating block for approximately 20 minutes, after which they were cooled to less than 100°C before adding 3.5 ml of potassium bromate (4.5% w/v). Each tube was then returned to the heating block and heated until the mixture had turned green, and all the bromine gas (brown vapour) had been driven off. The mixture was then cooled, transferred to a 50 ml volumetric flask, made up to volume with distilled water and mixed thoroughly by inversion. Ash remaining in the sample was allowed to settle out overnight before the liquid portion was transferred to sample bottles.

The liquid portion was assayed for chromium oxide content by atomic absorption using a nitrous oxide flame. The amount of chromium recovered was then expressed as a percentage of the original 1.5 g/kg



inclusion rate in the diet (equation 5.1) to determine the percentage digestibility of the diet.

**Equation 5.1. Digestibility Calculation.**

$$\% \text{ Digestion} = \frac{\text{Recovered Chromium (g/kg)} - 1.5 \text{ g/kg}}{\text{Recovered Chromium (g/kg)}}$$

**(ii) Free glucose and total starch**

Free glucose and total starch was measured according to the method described in section 3.3.1 and appendix 2.

**5.1.5. Statistical Analysis**

The data obtained for digesta and faecal samples were analysed by factorial analysis of variance, with wheat variety used as the treatment factor.

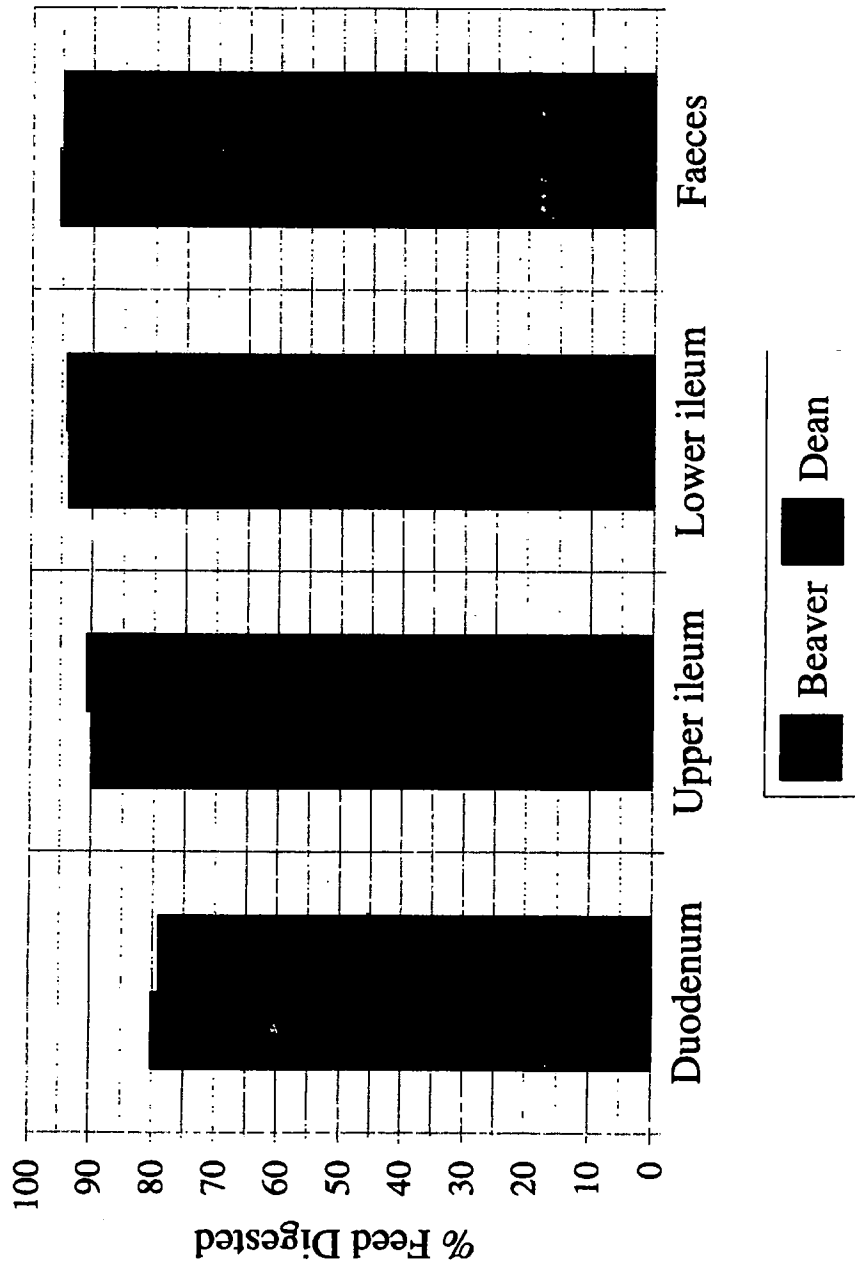
### 5.1.6. Results and Discussion

The results of the in vivo digestibility study are given in table 5.2, and displayed in graphs 5.1, 5.2 and 5.3 below. There were no significant differences between Dean and Beaver for any of the parameters. The results for dry matter digestibility from the chromium oxide analysis were all very similar and within the range 81.8 - 99.9 % reported by other workers (Rogel *et al.*, 1987; Annison, 1990; McNab, 1993; Nichol *et al.*, 1993), and the statistical analysis showed that there were no gross digestibility differences between the two diets.

**TABLE 5.2. BROILER *IN VIVO* DIGESTION OF DEAN AND  
BEAVER WHEAT SAMPLES HARVESTED IN 1990**

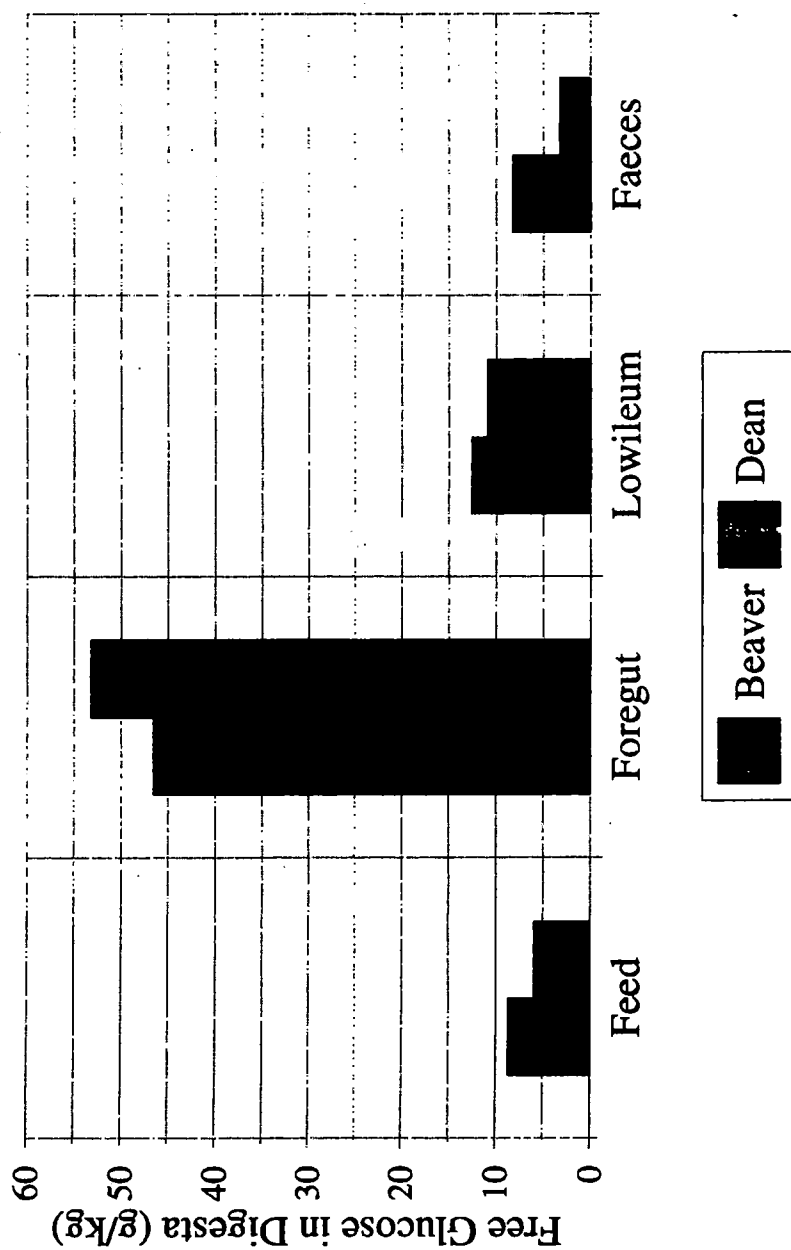
Wheat sample	Gut section	Dry Matter Digestibility (%)	Free glucose (g/kg)	Total starch (g/kg)
Beaver 1990	A	89.9	46.6	191
	B	94.0	12.6	60.9
	C	95.5	8.3	61.9
Dean 1990	A	90.8	53.3	238
	B	94.3	11.0	50.7
	C	94.9	3.3	65.5
SEM Variety (gut section A)		1.20	3.73	24.00
SEM Variety (gut section B)		0.44	1.16	5.73
SEM Variety (gut section C)		0.37	2.38	13.21
Residual DF		5	3	3

**GRAPH 5.1. IN VIVO DRY MATTER DIGESTIBILITY OF DEAN AND BEAVER WHEAT SAMPLES HARVESTED IN 1990 FED AS COMPLETE BROILER DIETS.**



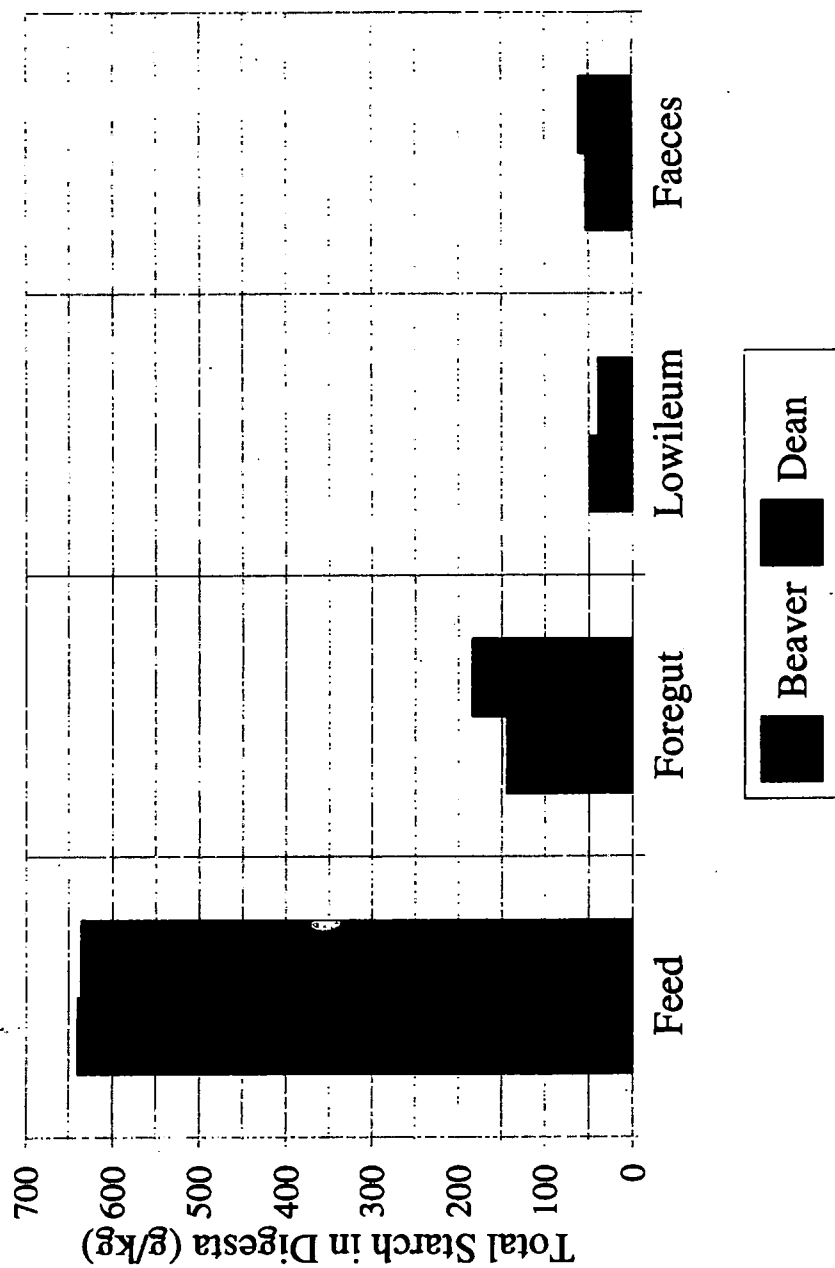
**GRAPH 5.2. FREE GLUCOSE FROM DIETS FORMULATED WITH DEAN AND BEAVER WHEAT**

**SAMPLES HARVESTED IN 1990 MEASURED *IN VIVO***



**GRAPH 5.3. DISAPPEARANCE OF STARCH FROM DIETS FORMULATED WITH DEAN AND BEAVER**

**WHEAT SAMPLES HARVESTED IN 1990 MEASURED *IN VIVO*.**



The variation between the replicate birds for both free glucose and total starch measurement for each diet was very high (CV=100% and 77% respectively for Dean and Beaver grand means), and there were many missing values in the dataset (6 missing values for the dry matter digestibility data, and 12 missing values for the free glucose and total starch data) due to insufficient sample volume, so any differences that may have existed were extremely difficult to detect. High variability in digestibility data recorded for young broilers has been reported by other researchers (Rogel *et al.*, 1987; Wiseman & Inborr, 1990; McNab, 1993), and has been especially associated with wheat diets.

Examination of the mean values of these measurements in the three gut sections did show some numeric differences ( $p>0.05$ ) that might have suggested digestibility differences between the two wheat samples. Birds fed the Dean-based diets had numerically higher mean levels of free glucose in gut section A (proximal ileum) than Beaver (graph 5.2), even though previous laboratory analysis had shown that samples of Dean had less free glucose than Beaver. Beaver digesta samples had more free glucose in sections B (distal ileum) and C (faecal material) than Dean. These observations suggested that the starch present in Dean tended to have been digested earlier and faster than that from Beaver resulting in

higher amounts of free glucose in the upper part of the ileum. This was supported by the results from the total starch analysis of the gut contents, where proportionally more starch from the Dean sample tended to disappear between gut sections A and B than did from the Beaver samples (graph 5.3). The higher levels of undigested starch present in the lower ileum for birds fed Beaver could have resulted in an increase in hind gut fermentation by caecal micro-organisms (Rogel *et al.*, 1987; Rose & Bedford, 1995). Hind gut fermentation and the corresponding release of nutrients is not as efficient as the digestive processes of the host, since it produces metabolites with different energetic characteristics to that of glucose (McDonald *et al.*, 1981). These compounds are more difficult for the host to absorb and utilise compared to glucose, and are strongly competed for by the bacteria that produced them (Bedford, 1995), two factors which may have affected the efficiency of nutrient assimilation and, thence, broiler growth.

Fig. . .

The majority of the starch had been digested (68% for Beaver and 79% Dean) by the time the digesta had reached the distal ileum (gut section B). The broilers also excreted some starch, an occurrence that had been previously been recorded in young broilers by McNab (1993). It was interesting to note that the starch content of the faeces (C) was



slightly higher than of the contents of the distal ileum (B), and this was attributed to the concentration of remaining undigested nutrients by removal of water, electrolytes, vitamins and volatile compounds from the faecal material in the lower gut (Larbier & Leclercq, 1994).

Variation between individual animals appears to have been of such a magnitude that a large number of animal replicates would have been needed to generate data that gave good statistical sample sizes for detection of differences. The method of digesta sample collection, which was not controllable even by the employment of the restricted feeding, and resulted in many missing digesta samples, also required improvement. Broilers tend to eat in a pattern of small, distinct, meals, and, even though precautions were taken to try to ensure uniform gut contents, there were visible differences in gut fill between individual animals. Finally, no allowance could be made for reverse peristalsis between the duodenum and gizzard, whereby digesta is sometimes moved back into the gizzard after it has passed into the duodenum. This action prolongs and increases digestion of feed, and would have varied between birds.

It was evident from the experiment that another, more controllable laboratory based method of assessing the carbohydrate digestion and glucose availability from the two varieties was needed.

## **5.2. IN VITRO RATE OF STARCH DIGESTION**

### **5.2.1. Objectives**

The objective of the experiment was to measure starch digestion by a more controllable *in vitro* method. The starch digestion characteristics of both wheat varieties, Dean and Beaver, from the three harvest years were determined using a laboratory method that simulated monogastric digestion. The rate of digestion was determined by measuring the rate of production of glucose from the wheat sample. Relationships between these measurements and the productive performance of the broiler chickens from the first growth experiment were examined.

### **5.2.2. Materials And Methods**

A laboratory model of *in vitro* monogastric carbohydrate digestion (Englyst *et al.*, 1992) was adapted and used to determine the rate of carbohydrate digestion in each of the six wheat samples (summarised in appendix 4). Approximately 0.8 g of milled sample was weighed into a 50 ml screw-top plastic tube and 50 mg of guar gum added. 10 ml of HCl containing 50 mg of pepsin (EC 3.4.23.1. Sigma cat. no. P-7000) in

solution was added to each tube, and vortex mixed thoroughly. The tubes were incubated in a water bath at 37°C for 30 minutes, during which time the carbohydrate enzyme solution was prepared.

To prepare sufficient enzyme for 24 samples and standards, 3g of pancreatin (Pancrex V, Paines & Byrne, Greenford, Middx) was weighed into each of eight plastic centrifuge tubes. Each portion was suspended in 20 ml of distilled water using a vortex mixer, and magnetically stirred for 10 minutes. Each tube was centrifuged at 1500 g for 10 minutes and 13.5 ml of the clearly supernatant removed into a flask containing 12 ml of amyloglucosidase solution (140 AGU/ml from 400 AGU/ml stock solution, EC 3.2.1.3, Novo Biolabs) and 8 ml of invertase (EC 3.2.1.26, 3000 EU/ml, BDH cat. no. 39020) and well mixed.

The samples were removed from the water bath and 5 glass marbles (1 cm in diameter), along with 10 ml of 0.25M sodium acetate, were added to each tube, which was shaken to disperse the contents. The tubes were replaced in the water bath at 37°C to equilibrate. The tubes were then removed from water bath one at a time, at 30 second intervals, and 5 ml of the carbohydrate enzyme mixture added. The tube was then immediately replaced in the bath, secured in a horizontal position, and

shaken at 160 strokes per minute, with a stroke length of 35 mm, so that the marbles moved freely back and forth within the tube to simulate the churning and peristaltic action of the gut. After each tube had been incubated for 15 minutes, 0.5 ml of the contents was removed into another tube containing 20 ml 66% ethanol and mixed well, which quenched the enzyme activity, preventing further digestion of starch. The tube containing the mixture was returned immediately to the water bath. This procedure was repeated at 30, 45, 60 and 120 minutes of incubation. The quenched portions were centrifuged at low speed for 5 minutes and the amount of glucose present in each portion was measured colorimetrically.

The amount of glucose present in the prepared portions was determined using a GOD-PAP glucose testing kit. 100  $\mu$ l from each sample, blank or standard was pipetted into a test tube. To this, 2 ml of the GOD-PAP reagent was added and the tubes were vortex mixed and placed in a 37°C water bath for 20 minutes. The absorbance of the standards and samples were measured in a spectrophotometer against the reagent blank at 510 nm. Glucose, in g/100 g sample, was calculated according to equation 5.2 below. The amounts of glucose released into solution at each of the five time points (along with the previously

determined free glucose content of the wheat samples (section 3.3) as the amount present at 0 min.) were plotted graphically and fitted to an exponential curve equation ( $y=a(1-e^{-cx})$ ) whereby y=appearance of glucose, a=intersection of curve with y axis, e=exponential, c=rate constant and x=time within a program of GENSTAT. The rate constant for each wheat sample was calculated for each replicate analysis and statistically compared as a two way analysis of variance, with wheat variety and harvest year as treatment factors.

#### **Equation 5.2. Calculation of Glucose Content**

$$\% \text{ Glucose} = \frac{A_t \times V_t \times C \times D}{A_s \times W_t} \times 100$$

Where:  $A_t$  = samples absorbance;  $V_t$  = total volume, C = concentration of standard (25 mg/ml);  $A_s$  = absorbance of standard;  $W_t$  = weight of sample (mg); D = dilution factor.

The experiment was repeated, but using samples containing different proportions of Dean and Beaver, ranging from 100% Dean, through 75% Dean and 25% Beaver, 50% of each variety, 25% Dean and 75% Beaver and 100% Beaver. The rate of starch hydrolysis was measured in exactly the same way in order to test whether or not the rate

of digestion was governed by any inhibitory factors such as  $\alpha$ -amylase inhibitors (Snow & O'Dea, 1981; Rogel *et al.*, 1987; Warchalewski *et al.*, 1989; McNab, 1993) in the more slowly digested varieties. If this had been the case, it would be expected that the rate would have been maintained at the lowest level whilst these factors were present, and then suddenly increase to the higher level when they were removed. Even if not completely inhibitory, a more gradual increase would be seen. If the rate was purely substrate (starch) dependant, then a linear relationship would be expected.

The *in vitro* rate of digestion results were compared with the broiler performance data from the first animal growth experiment by regression analysis, in order to investigate any relationship between the growth of the birds and starch digestion.

### **5.2.3. Results and Discussion**

The results are shown in table 5.3 below. There were large and highly significant differences ( $p < 0.001$ ) between the two wheat varieties. Dean had a 29% faster rate of *in vitro* digestion than Beaver over all three growing years (graph 5.4). There were no harvest year or variety x year

**TABLE 5.3. *IN VITRO* RATE OF STARCH DIGESTION FOR DEAN AND BEAVER WHEAT SAMPLES**

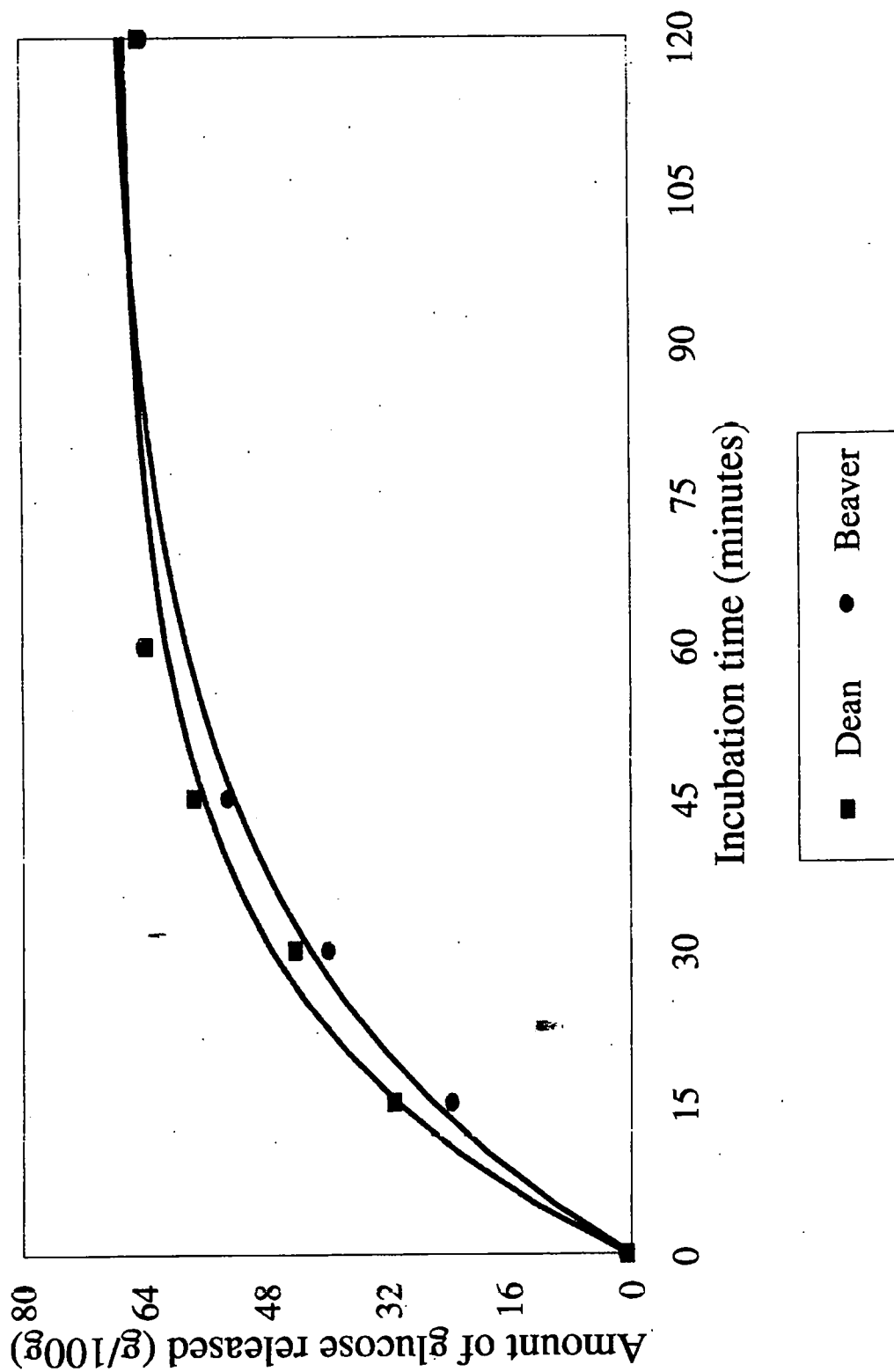
**HARVESTED IN 1990, 1991 AND 1992**

Variety	Year	Rate of Starch Digestion <i>in vitro</i> (mg/min/100g)
Beaver	1990	32.7
	1991	33.9
	1992	31.3
Mean		32.6
Dean	1990	41.2
	1991	42.5
	1992	42.6
Mean		42.1
SEM Variety		1.56***
SEM Year		1.90
SEM Variety x year		2.70
Residual D.F.		15



GRAPH 5.4. COMPARISON BETWEEN THE MEAN *IN VITRO* RATE OF DIGESTION FOR DEAN AND

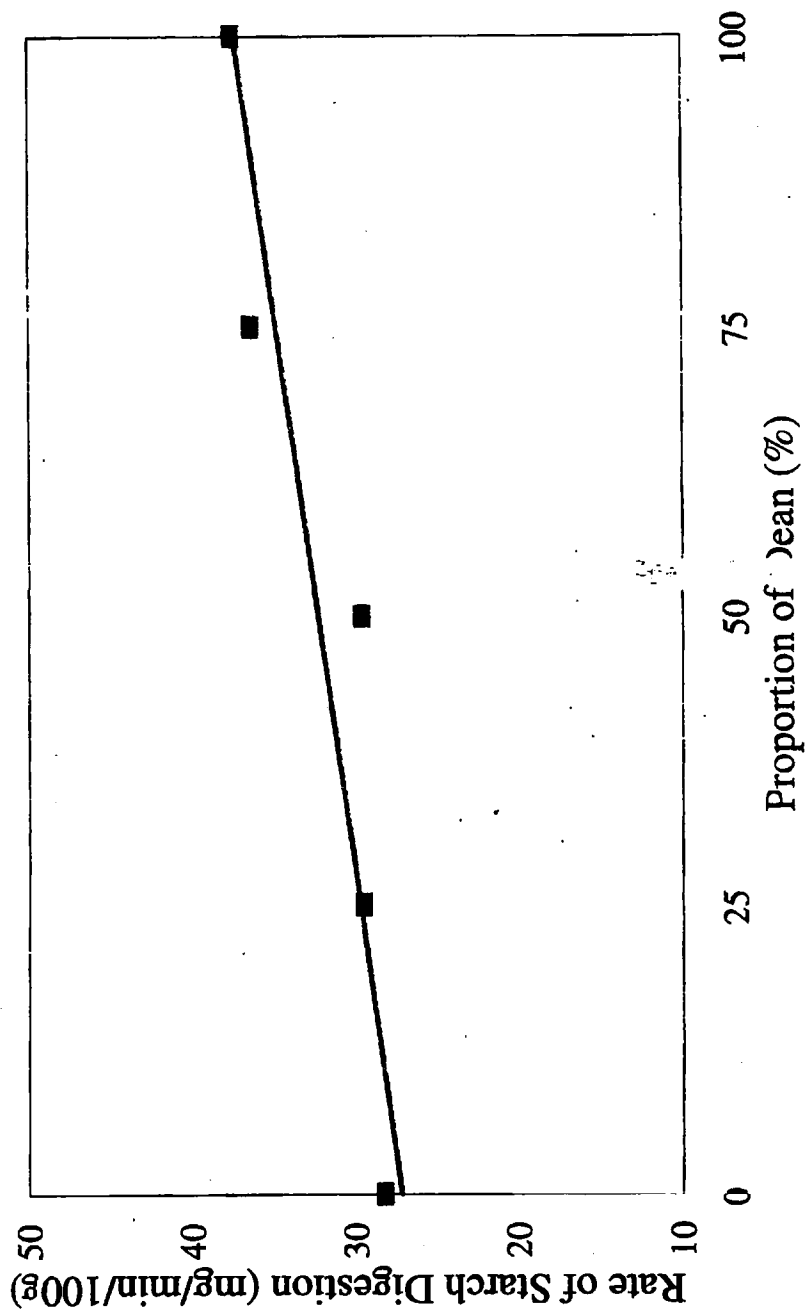
BEAVER WHEAT SAMPLES



**TABLE 5.4. IN VITRO RATE OF STARCH DIGESTION FOR DIFFERENT PROPORTIONS OF DEAN AND BEAVER WHEAT SAMPLES HARVESTED IN 1990, 1991 AND 1992**

Proportion of Dean (%)	Proportion of Beaver (%)	Rate of Starch Digestion <i>in vitro</i> (mg/min/100g)
100	0	37.7
75	25	36.4
50	50	29.7
25	75	29.5
0	100	28.2
SEM Mixture		0.001**
Residual D.F.		37

**GRAPH 5.5. RELATIONSHIP BETWEEN RATE OF STARCH DIGESTION AND PROPORTION OF DEAN AND BEAVER WHEAT SAMPLES HARVESTED IN 1990, 1991 AND 1992 IN TEST SAMPLE.**

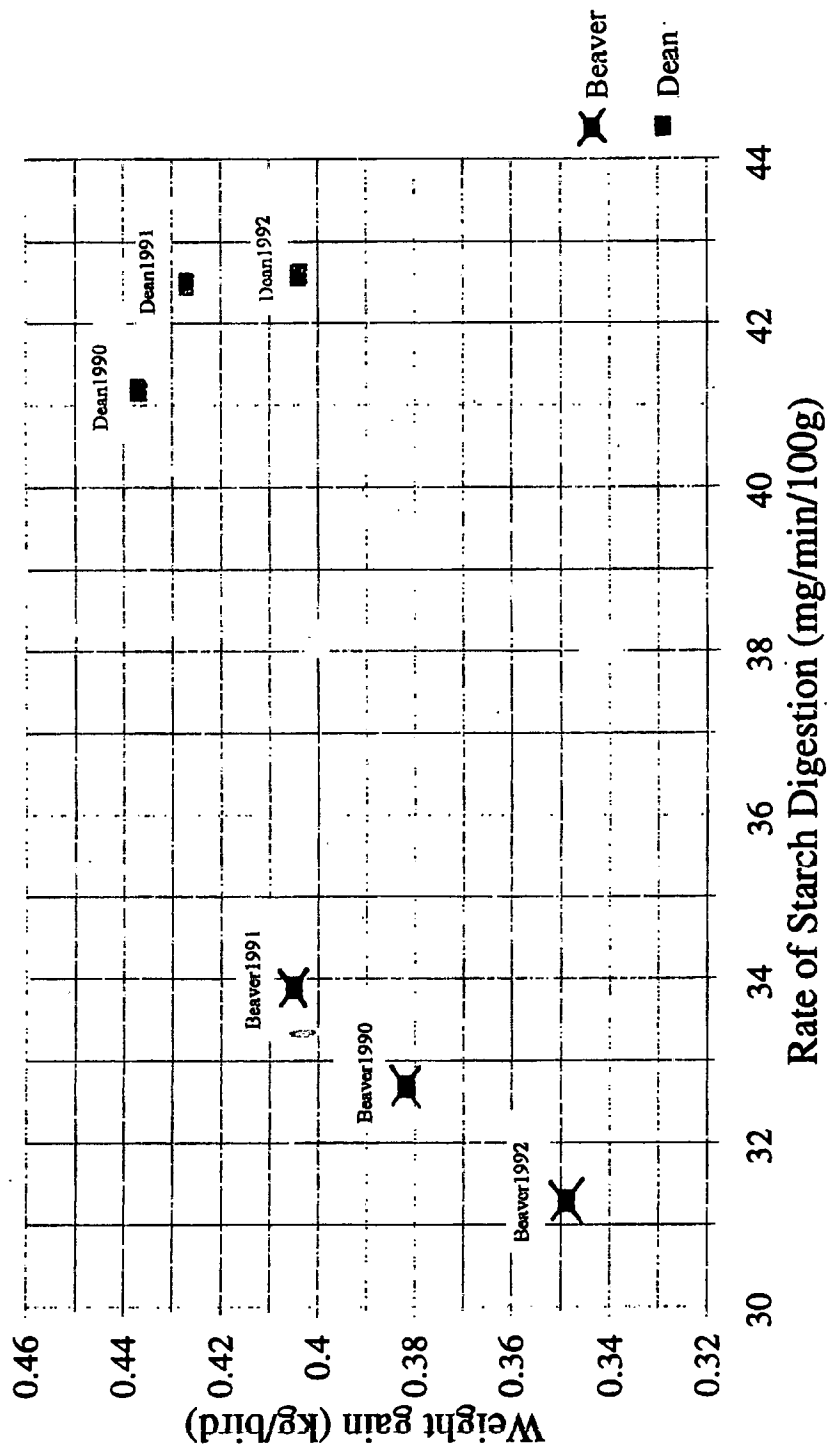


interactions for rate of digestion, therefore these differences were truly genotypic, and not controlled by environmental factors. The results of the experiment using the different proportions of the varieties (table 5.4), when expressed graphically (see graph 5.5) gave a highly significant ( $p < 0.001$ ) linear relationship ( $r = 0.574$ , 43 DF), which indicated that the rate of digestion was substrate (starch) dependant, and that there were no inhibitory factors contributing to the digestion processes. The substrate dependency of the *in vitro* rate of starch digestion was expected as wheat  $\alpha$ -amylase inhibitors have been found to be inactivated by the heat generated during milling (Snow & O'Dea, 1981) and by pepsin (Rogel *et al.*, 1987; McNab, 1993).

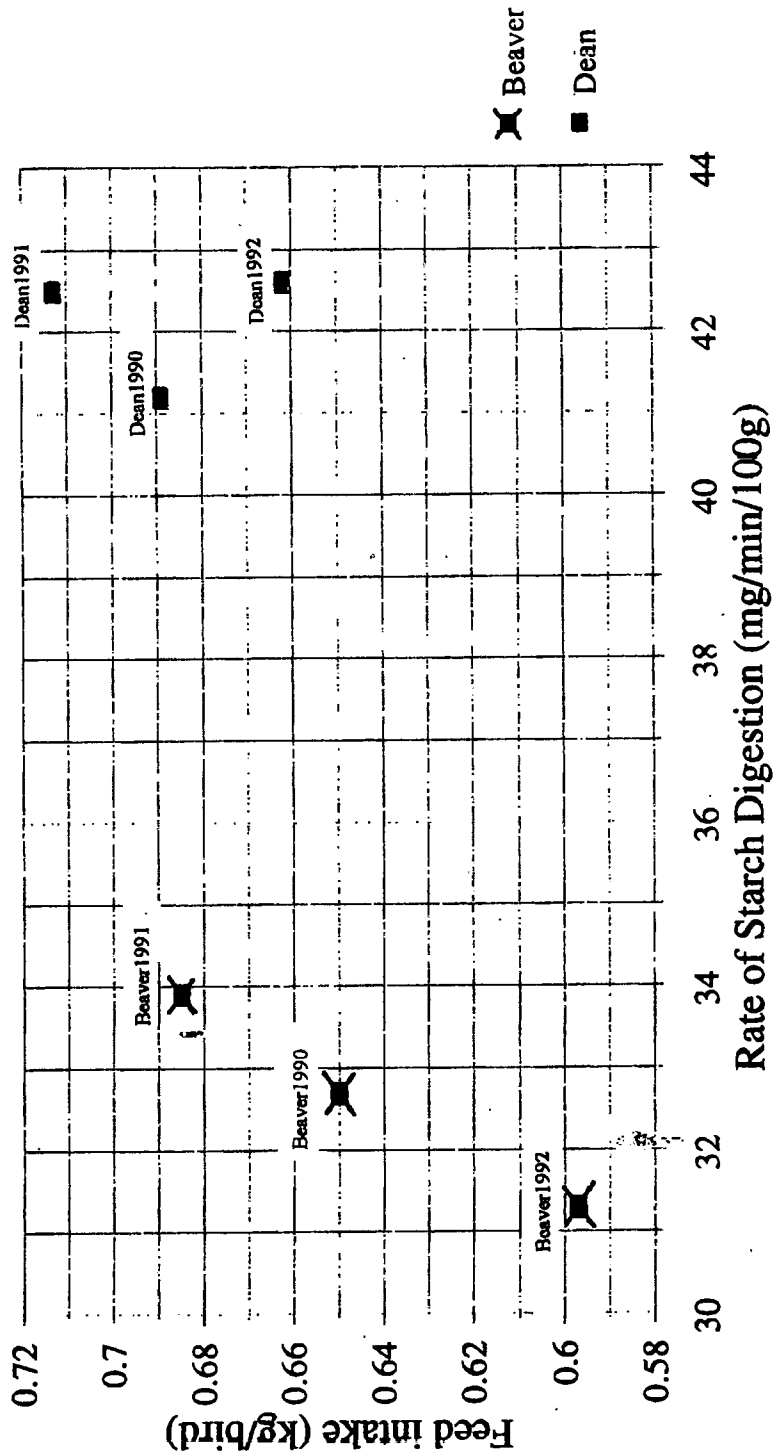
Regression analysis revealed that there was a trend of correlation between the rate of digestion and the broiler productive performance determined in the first broiler growth experiment (section 4.1). These relationships are shown in graphs 5.6, 5.7 and 5.8 below. Rate of starch digestion was related to broiler weight gain, with a correlation coefficient  $r = 0.808$  ( $p < 0.1$ , 4DF), but not significantly to feed intake or FCR, although the graphs indicated that there was a trend between these measurements and rate of digestion. Closer investigation revealed highly significant linear relationships between the rate of digestion and broiler

weight gain ( $r=0.998$ ,  $p<0.01$ ), feed intake ( $r=0.997$ ,  $p<0.01$ ) and FCR ( $r=0.999$ ,  $p<0.001$ ) for samples of the variety Beaver, but that there was a much more random relationship ( $r=0.776$ ,  $0.098$  and  $0.994$ , respectively;  $p<0.1$ , 1DF) between these parameters for Dean. This suggested that there may be a maximum rate of hydrolysis, at a rate of approximately 34 mg/min, after which other factors, such as rate of transport of glucose and other absorbable nutrients across the gut epithelium, limited the efficiency of assimilation and growth in the animal. The differences between rate of digestion for Dean and Beaver may have been a consequence of the type of starch present in each of the varieties (Rogel *et al.*, 1987). The particle size and amylose:amylopectin ratio results indicated that Dean probably contained more A-type starch granules than Beaver. This type of starch is thought to be more readily solubilised and more digestible by amylases than B- or C-type granules (Lund, 1984; Gruppen, 1996), and might account for the improvement in digestibility observed *in vitro* (Englyst *et al.*, 1992). The relationship between the rate of starch digestion and broiler productive performance may explain the differences in the nutritional value between UK wheat varieties and harvest years, and might have value as a method of estimating the nutritive value of wheat for broiler chickens.

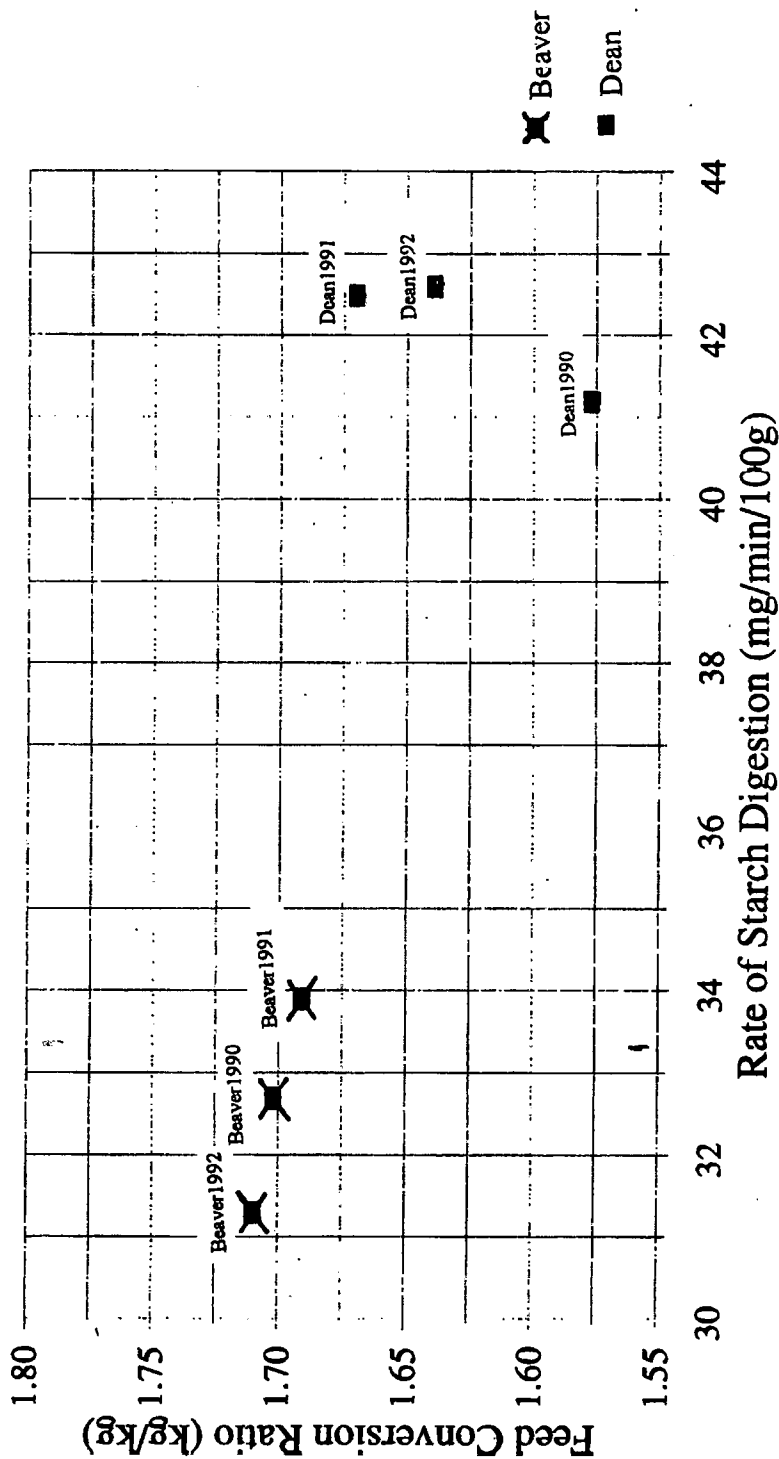
**GRAPH 5.6. RELATIONSHIP BETWEEN RATE OF STARCH DIGESTION AND WEIGHT GAIN OF 7 - 21 DAY OLD BROILER CHICKS FED SAMPLES OF DEAN AND BEAVER HARVESTED IN 1990, 1991 AND 1992**



**GRAPH 5.7. RELATIONSHIP BETWEEN RATE OF STARCH DIGESTION AND FEED INTAKE IN 7 - 21 DAY OLD BROILER CHICKS FED SAMPLES OF DEAN AND BEAVER HARVESTED IN 1990, 1991 AND 1992**



**GRAPH 5.8. RELATIONSHIP BETWEEN RATE OF STARCH DIGESTION AND FEED CONVERSION RATIO IN 7 - 21 DAY OLD BROILER CHICKS FED SAMPLES OF DEAN AND BEAVER HARVESTED IN 1990, 1991 AND 1992**





## **6. USING THE *IN VITRO* RATE OF STARCH DIGESTION TO ESTIMATE BROILER PRODUCTIVE PERFORMANCE.**

### **6.1. SCREENING WHEAT SAMPLES**

#### **6.1.1 Objectives**

The objective of the experiment was to investigate the magnitude of the variation in *in vitro* starch digestibility of different wheat varieties from different growing years.

#### **6.1.2. Materials And Methods.**

The *in vitro* rates of starch digestion of 16 different samples of pure wheat were analysed (see table 6.1). The wheat samples were of the varieties Apollo, Riband, Slejpner, Haven, Dean, Beaver, Brigadier and Rialto from three harvest years, 1992, 1993 and 1994, and grown as randomised field plots at Harper Adams Agricultural College. Randomised field plot samples were pooled in order to give a representative sample of each wheat variety from each growing year. The rate constant for each sample was measured in the same way as described

in section 5.2. The results were used to identify which harvest year had produced variety samples with the largest range of *in vitro* rate of digestion, and it was these samples that were used in the broiler experiment. Variety samples from different harvest years were not chosen, in order to eliminate any environmental influences at this stage.

### **6.1.3. Statistical Analysis**

The results were compared for analysis of variance, with wheat sample as the treatment factor.

### **6.1.4. Results**

The results of the analysis are shown in table 6.1. below. There were significant differences ( $p < 0.001$ ) in rate of starch digestion between the 16 wheat samples. It was decided, on the basis of these results, to use the wheat samples from 1994 harvest, since these showed a large but similar range of *in vitro* rates of starch digestion to those previously measured for Dean and Beaver from 1990, 1991 and 1992.

**TABLE 6.1. THE *IN VITRO* RATE OF STARCH DIGESTION OF  
16 WHEAT SAMPLES.**

Harvest Year	Variety	Rate of Starch Digestion <i>in vitro</i>
1992	Apollo	39.23
	Riband	44.50
	Slejpner	41.83
	Haven	38.42
	Range	38.42 - 44.50
1993	Dean	50.03
	Beaver	40.60
	Apollo	34.73
	Slejpner	48.90
	Riband	39.03
	Haven	33.98
	Range	34.73 - 50.03
1994	Dean	32.95
	Beaver	26.50
	Brigadier	39.53
	Rialto	30.18
	Riband	31.50
	Haven	29.08
	Range	26.50 - 39.53

## **6.2. RELATING THE DETERMINED *IN VITRO* RATE OF STARCH DIGESTION TO BROILER PERFORMANCE**

### **6.2.1. Objectives**

The validity of using the *in vitro* rate of starch digestion method as a determination of nutritive value of the wheat samples for broiler chickens was investigated. The main objective of the following experiment was to evaluate whether differences in the *in vitro* rate of starch digestion were related to differences in productive performance when the wheats were fed in complete diets to broiler chickens. The second objective was to determine the extent of influence of feed form (either meal-form or pelleted) on the *in vitro* rate of starch digestion and on the performance of the broilers.

### **6.2.2. Productive Performance Treatments**

Six variety samples - Dean, Beaver, Brigadier, Rialto, Riband and Haven - harvested in 1994 were used in the experiment. Diets were formulated with 700 g/kg wheat sample inclusion as described in section 2.2. After milling and mixing, the meal diets were divided in two, one of

which was cold pelleted, to give 12 diet treatments. Sub-samples of each of the diet treatments were taken for *in vitro* rate of starch digestion analysis (section 5.2) to identify effects of pelleting on starch availability and digestibility, and for comparison in regression analysis with the performance of the broilers.

### **6.2.3. Productive Performance Protocol**

A total of 192 male Ross broiler strain chicks were used in two identical experiments. Two time replicates had to be performed to ensure adequate replicate numbers of cages for each diet treatment, as facilities were limited to 48 cages each of which could only hold two broilers. At the beginning of the experiment a group of 96 one day-old broiler chicks were obtained and fed a conventional broiler starter diet and kept in a littered floor pen until 7 days old (average weight 0.158 kg per bird). The birds were then paired and housed in 48 cages. The cages measured 0.3m x 0.3m x 0.3m, with a floor mesh size of 1.5 cm x 1.5 cm and organised in two blocks. Dietary treatments were allocated to the cages according to a randomised block design, with a total of four cage replicates per diet for each experiment. The birds were given *ad libitum* access to feed and water from troughs measuring 11 cm x 11 cm and 10

cm x 8 cm respectively. They were maintained at a controlled temperature, starting at 30°C and declining to 25°C over the experimental feeding period, with a 23.5 hour lighting regime. The experiment lasted for 14 days, during which time weight gain, feed intake and feed conversion ratio (FCR) were measured. Half way through the first experiment, the next group of 96 one day-old broilers were placed on a conventional starter diet, in readiness for the second experiment. At the end of the experiment, the next set of 7 day old broiler chicks were placed in the cages, and the experiment repeated.

#### **6.2.4. Statistical Analysis**

The results of the productive performance trial and the *in vitro* rate of starch digestion of the complete diets were analysed as a factorial analysis of variance. Wheat variety and diet form (meal or pellet) were designated treatment factors, and the experimental data recorded from the broiler chickens blocked by experiment number, cage level and diet replicate, and analysed on an average bird per cage (rather than an individual bird) basis. Rate of starch digestion data was blocked by time replicate. Linear regression analysis was performed to ascertain any relationships between broiler performance parameters and *in vitro* rate of

starch digestion, and was also divided by diet form to further investigate the differences in these relationships between meal and pelleted diets.

#### 6.2.5. Results

The results of the experiment are displayed in table 6.2 below. There were significant differences in weight gain ( $p < 0.05$ ) and *in vitro* rate of starch digestion ( $p < 0.001$ ) between the six wheat varieties studied. The rates of digestion were sometimes slightly lower than those recorded either in the screening or the previous experiment (section 6.1 and 5.2 respectively), as the rate was determined upon the whole diet rather than the pure wheat samples. There was a large significant interaction ( $p < 0.001$ ) between wheat variety and diet form for broiler weight gain and *in vitro* rate of starch digestion, which suggested that the variety effects upon these two parameters were influenced by processing. In order to investigate how the significant differences between varieties altered due to the effects of processing, the meal and pellet data was analysed separately to obtain p-values for varietal differences in broiler growth and *in vitro* rate of starch digestion within each diet form. This analysis identified whether the significant varietal differences were

confined to only one diet form, or whether level of significance altered between the two forms.

When the meal diets were analysed in isolation, significant varietal differences were observed for broiler weight gain ( $p < 0.01$ ) with the variety Brigadier giving the largest broiler weight gains, and Beaver giving the smallest. Feed intakes significantly differed ( $p < 0.05$ ) between the varieties fed as meal diets, with Brigadier giving highest and Riband the lowest results, which indicated that increased feed intake might have caused the larger weight gains with Brigadier. There were no significant differences between the varieties for FCR, but there were highly significant varietal differences for *in vitro* rate of starch digestion ( $p < 0.001$ ), mirroring the weight gain rankings with Brigadier having the fastest and Beaver the slowest rates. This indicated that the rate of digestion could have been related to the weight gain of the broilers.

There were significant variety differences in weight gain for the birds fed the pelleted diets, however the level of significance was lower ( $p < 0.05$ ) than the differences recorded for the meal diets. There were no significant differences between either feed intake or broiler FCR.



**TABLE 6.2. THE RATE OF STARCH DIGESTION AND PRODUCTIVE PERFORMANCE OF 7-21 DAY OLD**

**BROILERS FED SIX WHEAT VARIETIES AS COMPLETE DIETS IN MEAL OR PELLETTED FORM.**

Variety	Diet Form	Weight gain (kg/bird)	Feed Intake (kg/bird)	FCR (kg/kg)	Rate of starch digestion <i>in vitro</i> (mg/min/100g)
Dean	Meal	0.529	0.819	1.548	28.17
Beaver		0.497	0.807	1.630	26.74
Brigadier		0.550	0.891	1.641	33.21
Rialto		0.506	0.826	1.634	27.81
Riband		0.505	0.794	1.573	30.54
Haven		0.503	0.822	1.637	29.78
Mean		0.515	0.827	1.610	29.38
Dean	Pellet	0.535	0.835	1.565	34.14
Beaver		0.577	0.854	1.478	34.33
Brigadier		0.536	0.834	1.563	43.18
Rialto		0.496	0.798	1.610	38.14
Riband		0.551	0.879	1.593	38.66
Haven		0.530	0.832	1.568	37.84
Mean		0.538	0.839	1.563	37.72
SEM Variety	SEM Variety x Diet form	0.0087*	0.0160	0.0251	0.278***
SEM Diet form		0.0050**	0.0093	0.0145*	0.166***
SEM Variety x Diet form		0.0122***	0.0227	0.0356	0.406***
DF		47	47	47	95

\* p<0.05; \*\* p<0.01; \*\*\* p<0.001

The significance level of the variety differences for pelleted diets *in vitro* rate of starch digestion remained at the same level as for the meal ( $p < 0.001$ ).

Pelleting the feed had an impact on the broiler weight gain ( $p < 0.01$ ) and FCR ( $p < 0.05$ ) for each of the wheat varieties. The interaction between variety and diet form ( $p < 0.001$ ) for weight gain manifested itself as a change in the ranking of the varieties. In contrast to the results obtained from the broiler chicks fed the meal diets, Beaver gave the highest, and Rialto the lowest weight gain. The weight gains of the broilers fed Dean, Beaver, Riband and Haven all improved when pelleted, although those fed Brigadier and Rialto grew less well than those fed the same varieties in meal form. The lack of a variety x diet form interaction for FCR indicated that pelleting consistently improved broiler feeding efficiency.

Pelleting the diets significantly improved the *in vitro* rate of starch digestion ( $p < 0.001$ ) for all six wheat varieties. The large significant ( $p < 0.001$ ) interaction between variety and diet form showed that this improvement was not uniform. When the percentage increase in *in vitro* rate of starch digestion was calculated a range of 21 - 37% was seen,

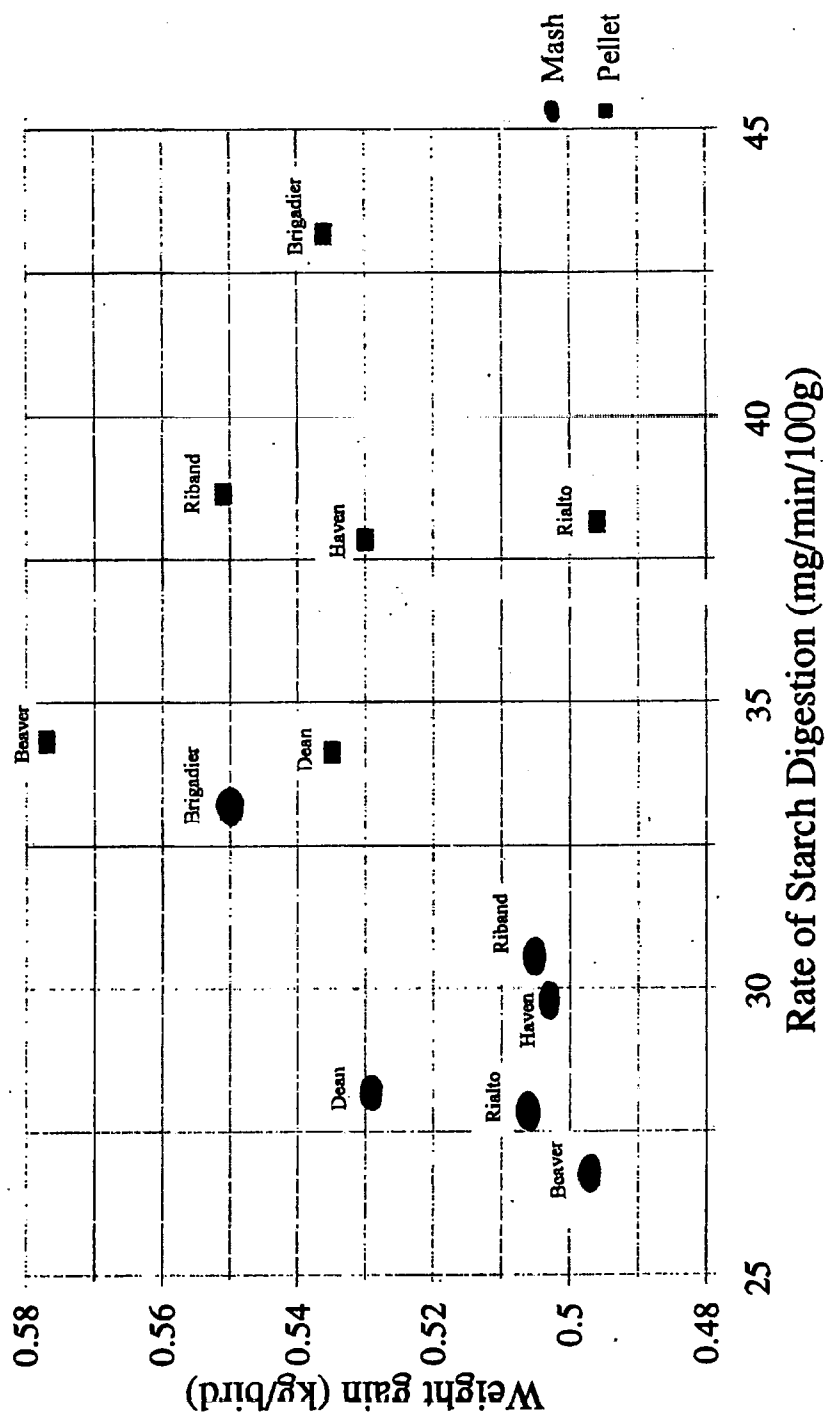
with Dean having the lowest and Rialto the highest improvement. Haven, Riband, Beaver and Brigadier showed 27%, 27%, 29% and 30% improvement respectively. Pelleting is a method commonly used to improve the digestibility of poultry diets (Larbier & Leclercq, 1994), however the extent of this effect appeared to vary with respect to wheat variety in this experiment.

Regression analysis was performed on those parameters that showed significant differences for wheat variety and diet form, i.e. weight gain and rate of starch digestion, to investigate the influence of one on the other. There were no significant relationships between broiler performance and *in vitro* rate of starch digestion ( $r=0.408$  for weight gain;  $r=0.315$  for feed intake and  $r=-0.023$  for FCR) when all 12 diet treatments were compared graphically (graphs 6.1, 6.2 and 6.3). When the data was divided by diet form and analysed, a trend ( $r=0.686$ ;  $p>0.1$ ) was found between the *in vitro* rate of starch digestion and both broiler weight gain and feed intake for meal diets which was not found for the pelleted diets ( $r=-0.310$  for weight gain and  $r=0.099$  for feed intake). This relationship was, however, due to one outlying value (Brigadier), removal of which negated the trend. No significant relationship could be found between the *in vitro* rate of starch digestion and FCR ( $r=0.154$  for

meal diets and  $r=0.436$  for pelleted diets) even when the data was separated by diet form.

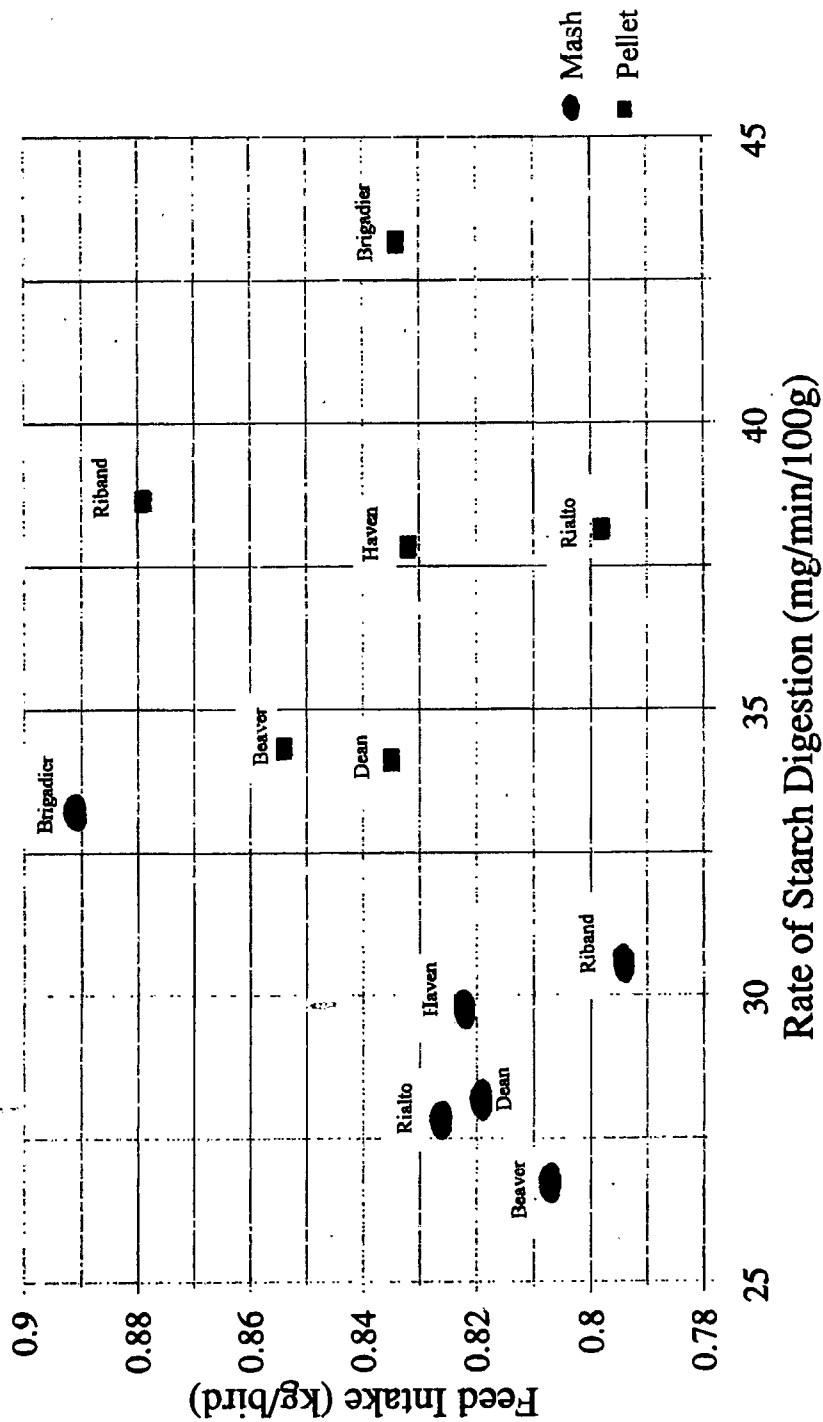
**GRAPH 6.1. RELATIONSHIP BETWEEN THE RATE OF STARCH DIGESTION AND WEIGHT GAIN FOR 7-21 DAY OLD BROILERS FED SIX WHEAT VARIETIES AS COMPLETE DIETS IN MEAL OR PELLETTED**

**FORM**

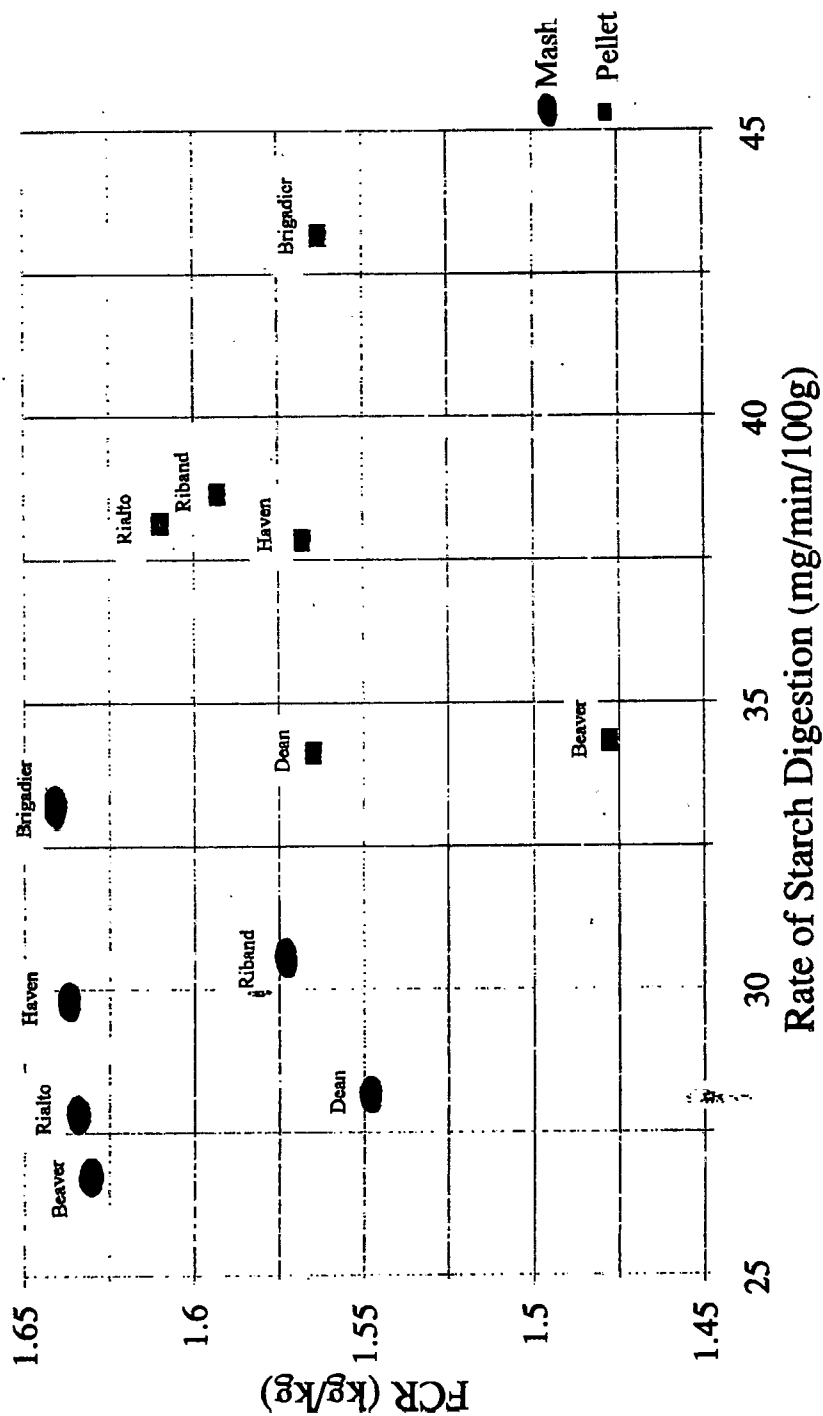


**GRAPH 6.2. RELATIONSHIP BETWEEN THE RATE OF STARCH DIGESTION AND FEED INTAKE FOR 7-21 DAY OLD BROILERS FED SIX WHEAT VARIETIES AS COMPLETE DIETS IN MEAL OR PELLETTED**

**FORM**



**GRAPH 6.3. RELATIONSHIP BETWEEN THE RATE OF STARCH DIGESTION AND FEED CONVERSION**  
**RATIO FOR 7-21 DAY OLD BROILERS FED SIX WHEAT VARIETIES AS COMPLETE DIETS IN MEAL OR**  
**PELLETED FORM**



### 6.2.6. Discussion

The results of the experiment indicated that there were varietal differences in both *in vitro* rate of starch digestion and broiler weight gain. The form of the diet significantly influenced the growth and FCR of the broilers and the *in vitro* rate of starch digestion.

The heat generated due to friction during the formation of the pellet can raise the temperature of the feed to approximately 70°C, which improves starch gelatinisation (Snow & O'Dea, 1981; Rogel *et al.*, 1987; Larbier & Leclercq, 1994). Most likely these effects caused the increase in *in vitro* rate of digestion (Snow & O'Dea, 1981; Rogel *et al.*, 1987; McNab, 1993). Certainly the significant improvements recorded overall for the pelleted form of the diets in weight gain and FCR indicated that the broilers were able to digest and utilise more nutrients from pelleted diets.

The varieties that showed a reduction in broiler performance due to pelleting, either for weight gain or FCR, may have been due to a number of reasons. Heat of processing may gelatinise starch to a level whereby it crystallises upon cooling. This crystalline starch is known as retrograded



starch and is highly resistant to digestion (Colonna *et al.*, 1992; Englyst *et al.*, 1992) and may have been the cause of loss of broiler performance (McNab, 1993). Heat may also cause increased solubilisation of anti-nutritive factors (such as NSP) which indirectly interferes with digestion by increasing digesta viscosity (McCracken *et al.*, 1993; Bedford & Morgan, 1995) within the digestive tract. Measurements of retrograded starch and soluble NSP were not made during this experiment and so no conclusions can be drawn for the extent of such changes due to heat. Increases in the levels of retrograded starch would have resulted in more starch being fermented by the hind gut microflora (Bedford, 1992; Annison, 1993). It has been observed that the slower the rate of digestion by the bird's own enzymes, the higher the bacterial activity in the caeca, due to increased nutrient availability for fermentation (Bedford 1995). Changes in bacterial numbers within the gut, and the less efficient uptake and metabolism of the resulting volatile fatty acids by the broiler could all have influenced the performance observed in this experiment.

The most important difference between the results of this experiment and the experiment described in section 5.2 was the lack of significant differences in FCR between wheat varieties, and the absence of significant relationships between rate of starch hydrolysis and broiler

performance. This indicated that the rate of *in vitro* starch digestion could not be used as an accurate predictor of the nutritional quality of wheat for growing broiler chickens.

## 7. GENERAL SUMMARY

The first broiler feeding experiment showed that there were differences in weight gain and feed conversion ratio between birds fed the two wheat varieties, Dean and Beaver. This confirmed the variety effects on broiler growth previously observed by Rose *et al.* (1993) and Salah Uddin *et al.* (1996). These differences were also evident in the second feeding experiment, which demonstrated that variations in the nutritive value of the two wheat varieties were consistent over three age groups corresponding to the whole growing period of commercial broiler chickens. The weight gains of the birds fed Dean were 12% and 9% greater than Beaver, with a corresponding improvement in FCR of 4% and 5%, in the first and second feeding experiments respectively. These differences amounted to commercially important variations in animal growth and feeding efficiency. Harvest year effects were smaller in comparison with variety effects, with only one significant variety x year interaction observed for diets formulated with Dean and Beaver from 1990, 1991 and 1992, as used in the first feeding experiment. All the wheat samples used were acceptable in terms of Hagberg falling number and specific gravity for feed grade classification in the UK.

There were no consistent differences in the AMEn or TMEn values between the wheat varieties, which agreed with the findings of previous studies. McNab (1991; 1996) could not determine varietal differences in TME value, even for the wheat variety Slejpner that had been identified as being of low nutritional quality by commercial broiler growers. Dean harvested in 1990 had a particularly high TMEn value, which led to a significant year effect ( $p < 0.001$ ) and variety x year interaction ( $p < 0.05$ ). There were no significant correlations between AMEn or TMEn value and broiler weight gain or FCR, which concurred with the observations of Rose & Bedford (1995). The chemical composition and quality characteristics of the wheat samples were not related to the broiler performance, with the exception of a correlation between FCR and HFN, as previously observed by Rose *et al* (1993). Wheat NSP, which had been previously related to poor broiler growth (Wiseman & Inborr, 1990; Annison, 1993; Wiseman, 1993) was not related to the changes in broiler productive performance in the broiler feeding experiments. The relationship between NSP and broiler growth and ME values appears to be confined to birds that receive a high ( $> 800$  g/kg inclusion) wheat diet (Wiseman & Inborr, 1990; McNab, 1993) or wheat samples that exhibit low AME characteristics (Annison & Johnson, 1989; Annison, 1993; Wiseman, 1993).

Starch content and availability appeared to be constant between the two varieties Dean and Beaver, and as the ME and chemical composition of the wheat could not be used to determine nutritive quality, other parameters had to be investigated. The relationship between HFN and FCR indicated that the solubility of the wheat starch (Hagberg, 1960, 1961) may have influenced broiler feeding efficiency. It was for this reason that the rate of starch digestion characteristics of Dean and Beaver were examined.

Wheat is generally considered to be well digested (McNab, 1993; Annison, 1990) however *in vivo* digestibility may be variable (Rogel *et al*, 1987; Nichol *et al.*, 1993). Higher rates of digestion may affect feed throughput and hence, feed intake; alternatively, if more starch was digested in the proximal rather than the distal ileum, then less would be available for hind gut fermentation, resulting in improved feed utilisation.

An *in vitro* model of monogastric digestion was used to determine the rate of starch digestion. There were significant varietal differences ( $p < 0.001$ ) in the *in vitro* rate of starch digestion whereby Dean had a much faster rate of digestion than Beaver. These differences were substrate

dependant, and were therefore not caused by any inhibitory factors in the wheat. A relationship was found between the *in vitro* rate of starch digestion and broiler weight gain ( $r=0.808$ ,  $p<0.1$ ). Closer investigation revealed that *in vitro* rate of starch digestion for samples of the more slowly digested variety (Beaver) were linearly related to broiler weight gain ( $r=0.998$ ,  $p<0.01$ ), feed intake ( $r=0.997$ ,  $p<0.01$ ) and FCR ( $r=0.999$ ,  $p<0.001$ ).

Multiple regression analysis of the relationship between *in vitro* rate of starch digestion of Dean and Beaver from 1990, 1991 and 1992 harvest years and broiler weight gain from the first feeding experiment was conducted (table 7.1). The chemical and carbohydrate composition, quality and ME values of the wheat samples were added into the analysis model. The only measurement that significantly reduced the residual sum of squares was the TMEn value. It was interesting that TMEn, and not AMEn, affected the relationship, as this lends further evidence that the AME protocol is confounded by feed intake and faecal collection errors (McNab, 1993, 1996), endogenous secretions, viscous gut conditions and interactions with hind gut microflora (Rose *et al.*, 1992; Wiseman, 1992; McNab, 1996; Rose & Bedford, 1995). These factors may not affect

TMEn determinations since the digestive tract is completely cleared out during the fasting period, and feed intake is carefully controlled.

There was the possibility that the differences and correlations observed between *in vitro* rate of starch digestion and broiler growth were not causal relationships, but merely a consequence of the variety with the higher *in vitro* rate of starch digestion also happening to result in improved broiler growth. A final feeding experiment using six wheat varieties from a different harvest year (1994) was conducted in order to verify the relationship. This experiment also investigated the effect of pelleting on the varietal differences in broiler performance and *in vitro* rate of starch digestion. The results indicated that there was no relationship between the rate of digestion and broiler growth, even though significant varietal differences remained for broiler weight gain ( $p < 0.05$ ) and *in vitro* rate of starch digestion ( $p < 0.001$ ). The relationship observed in the earlier experiments appeared to have been an anomaly of the two varieties, Dean and Beaver. Pelleting the diets caused the broiler weight gain rankings of the wheat varieties to change, an effect which was attributed to genotypic differences in the degree of retrograded starch formation (Rogel *et al.*, 1987; Colonna *et al.*, 1992; Englyst *et al.*, 1992; McNab, 1993). Pelleting also improved *in vitro* rate of starch digestion, an effect previously

reported by Rogel *et al.* (1987) using a more crude *in vitro* analysis of starch digestibility. The differences in rate of digestion, along with the changes in broiler growth, warrant further investigations into the effects of processing on digestibility and broiler growth.



**TABLE 7.1 THE LINEAR RELATIONSHIP BETWEEN PARAMETERS OF WHEAT-FED BROILER PRODUCTIVE PERFORMANCE (7-21 DAYS OLD) AND THE RATE OF STARCH DIGESTION, OR THE RATE WITH THE TME IN A MULTIPLE REGRESSION ANALYSIS**

Dependant Variable	Intercept (±SE)	Regression Coefficients (±SE)		Standard error of observations	% variance accounted for
		<i>in vitro</i> rate of starch digestion (mg/min/100g)	TME <sub>n</sub> (MJ/kg of dry matter)		
Weight gain (kg/bird)	0.2186(±0.0670)	0.00487 (±0.00178)	-	0.0210	56.5
	-1.170(±0.429)	0.00376(±0.00103)	0.0977(±0.0301)	0.0114	87.2
Feed intake (kg/bird)	0.468(±0.104)	0.00529(±0.00276)	-	0.0325	34.9
	-1.01(±1.12)	0.00411(±0.00269)	0.1043(±0.0788)	0.0299	45.2
FCR (kg/kg)	1.931 (±0.119)	-0.00712 (±0.00316)	-	0.0373	44.8
	3.93 (±1.13)	-0.00552 (±0.00271)	-0.1408(±0.0794)	0.0301	64.1

Residual DF=4 (single linear regression) and 3 (multiple regression analysis)

## 8. GENERAL CONCLUSIONS

- (i) A comparison of the nutritional quality of the two wheat varieties, Dean and Beaver, showed that there were significant and commercially important differences in weight gain, feed intake and feed conversion ratio when they were included at 700 g/kg in diets formulated for growing broiler chickens. These differences were continuous over the commercial growing period of broilers.
- (ii) There were significant differences in growth between broiler chickens fed wheat samples harvested in 1990, 1991 and 1992, however these were small in comparison with the variety effects.
- (iii) There were no significant differences in AMEn or TMEn values between the varieties Dean and Beaver.
- (iv) Dean had a significantly higher Hagberg falling number and specific gravity than Beaver; these characteristics were significantly related to broiler feed conversion ratio and weight gain respectively.

- (v) Dean had a significantly faster *in vitro* rate of starch digestion than Beaver, an effect that was found to be substrate dependant and not influenced by any inhibitory factors.
- (vi) Although multiple regression analysis including both *in vitro* rate of starch digestion and TMEn of the wheat samples explained 87% of the variance in broiler weight gains between the three harvest year samples of the varieties Dean and Beaver, a further experiment with six UK wheat varieties grown in 1994 showed no relationship between *in vitro* rate of starch digestion and broiler growth.

## REFERENCES

Abdul-Baki, A.A. & Anderson, J.D. (1972) in Seed biology Vol.2. Ed. T.T.Kozlowski. pp.283-315. Academic Press, New York & London.

Albertini, M. (1995) Particle size of flour and wheat quality. Personal communication.

Alpine, A.L. & Sheshukova, T.A. (1992). Distribution of disaccharidase activity in the chick intestinal villus. *Latvijas Zinatnu Akademijas Vestis. B Dala, Dabaszinibas. No. 4*, 72-75.

Aman, P. & Graham, H. (1990). Chemical evaluation of polysaccharides in animal feeds. in Feedstuff Evaluation. Eds.: J. Wiseman & D.J.A.Cole. pp. 161-178. Butterworths, London

Annison, E.F. (1974). in Energy Requirements of Poultry eds. T.R.Morris & B.M.Freeman. pp.135-149. British Poultry Science Ltd., Edinburgh.

Annison, G. & Johnson, R.J. (1989). Relationship between AME, Starch digestibility and pentosan levels in rice, sorghum and wheat diets for broiler chickens. *Proceedings of the Australian Poultry Science Symposium*. pp. 79-82. Sydney, Australia.

Annison, G. (1989). Determination of AME of wheat using gnotobiotic chickens. Recent advances in Animal Nutrition in Australia. Ed. Farrell. Armidale, Australia

Annison, G. (1990). Polysaccharide composition of Australian wheats and digestibility of their starches in broiler chicken diets. *Australian Journal of Experimental Agriculture* **30**: 183-186

Annison, G. (1992). Commercial enzyme supplementation of wheat-based diets raises ileal glycanase activities and improves apparent metabolisable energy, starch and pentosan digestibilities in broiler chickens. *Animal Feed Science and Technology*. **38**: 105-121.

Annison, G. (1993). The role of wheat non-starch polysaccharides in broiler nutrition. *Australian Journal of Agricultural Research*. **44**: 405-422.

Antoniou, T. & Marquardt, R.R. (1981). Influence of rye pentosans on the growth of chicks. *Poultry Science*. **60**: 1898-1904.

Antoniou, T., Marquardt, R.R. & Cansfield, P.E. (1981). Isolation, partial characterisation and antinutritional activity of a factor (pentosans) in rye grain. *Journal of Agricultural Food Chemistry*. **29**: 1240-1247.

AOAC (1990). Official Methods of Analysis. AOAC, Virginia, USA.

ARC/MRC (1974). Food and nutrition research. Chapter 1. HMSO, London.

Baghel, R.P.S. & Pradhan, K. (1989). Effect of dietary protein and energy on broiler carcass composition. *Indian Veterinary Journal*. 66: 9, 847-850.

Ball, J.A. (1988). Variability of raw materials. *The Feed Compounder*. 8: 1, 18-23.

Bechtel, D.B., Zayas, I., Dempster, R. & Wilson, J.D (1993). Size distribution of starch granules isolated from hard red winter wheat and soft red winter wheats. *Cereal Chemistry* 70: 2, 328-240

Bechtel, D.B., Zayas, I., Kaleikau, I. & Pomeranz, Y. (1990). Size-distribution of wheat starch granules during endosperm development. *Cereal Chemistry* 67: 1, 59-63.

Bedford, M.R. (1992). The effect of dietary enzymes on digestion in poultry. *Feed Compounder*. 12: 10.

Bedford, M.R. (1995). Recent developments in the practical application of feed enzymes in poultry feed. *Forum's Nutrition Forum Poultry Proceedings*. Forum, London.

Bedford, M.R. & Classen, H.L. (1992). Reduction of intestinal viscosity through manipulation of dietary rye and pentosanase concentration is effected through changes in the carbohydrate composition of the intestinal aqueous phase and results in improved growth rate and food conversion efficiency of broiler chicks. *The Journal of Nutrition*. **122**: 3, 560-569.

Bedford, M.R. & Morgan, A.J. (1996). The use of enzymes in poultry diets. *World's Poultry Science Journal*. **52**: 61-68.

Behall, K.M., Scholfield, D.J., Yuhaniak, I. & Canary, J. (1989). Diets containing high amylose vs amylopectin starch: effects on metabolic variables in human subjects. *American Journal of Clinical Nutrition*. **49**: 337-344.

Bengtsson, S. & Aman, P. (1990). Isolation and chemical characterisation of water-soluble arabinoxylans in rye grain. *Carbohydrate polymers* **12**: 267-277.

Berlyn, G.P. (1972). in Seed biology Vol.1. Ed. T.T.Kozlowski. pp.229-232. Academic Press, New York & London.

Bertoft, E. & Manelius, R. (1992). A method for the study of the enzymic hydrolysis of starch granules. *Carbohydrate Research*. **227**: 269-283.

Best, S. & Muller, R. (1991). Use of the Hagberg falling number apparatus to determine malt and barley quality. *Journal of the Institute of Brewing* **97**: 273-278.

Bewley, J.D. & Black, M. (1978). Physiology and biochemistry of seeds. Vol. 1 chapter 2. Springer-Verlag, New York.

Bhatnagar, S.P. & Johri, B.M (1972). in Seed biology Vol.1. Ed. T.T.Kozlowski. pp.106-118. Academic Press, New York & London.

Blake, J.P. & Potter, L.M. (1987). Nitrogen, energy and weight losses associated with drying and grinding of poultry feed and excreta. *Poultry Science*. **66**: 112-119.

Bonnet, S. (1994). The metabolisable energy of two wheat varieties when given as whole grains or in a complete mixed feed. Personal Communication.

Brenes, A., Smith, M., Guenter, W. & Marquardt, R.R. (1993). Effect of enzyme supplementation on the performance and digestive tract size of broiler chickens fed wheat and barley based diets. *Poultry Science*. **72**: 9, 1731-1739.

Brennan, C.S., Sulaiman, B.D., Schofield, J.D. & Vaughn, J.G. (1993). The immunolocation of friabilin and its association with wheat endosperm texture. *Aspects of Applied Biology* **36**: 69-73.



Brinson, K. & Dey, P.M. (1982). Polysaccharides containing xylose, arabinose and galactose in higher plants in Biochemistry of Seed Storage Carbohydrates in green Plant eds. P.M.Dey and R.A.Dixon. Chapter 10. Academic Press, London.

Brooks, A., Jenner, C.F. & Aspinall, D. (1982). Effects of water deficit on endosperm starch granules and on grain physiology of wheat and barley. *Australian Journal of Plant Physiology* 9: 423-436.

Campbell, G.L., Rossnagel, B.G., Classen, H.L. & Thacker, P.A. (1989). Genotypic and environmental differences in extract viscosity of barley and their relationship to its nutritive value for broiler chickens. *Animal Feed Science and Technology* 26: 221-230.

Campbell, G.L., Salmon, R.E. & Classen, H.L. (1985). Prediction of metabolisable energy of broiler diets from chemical analysis. *Poultry Science* 65: 2126-2134.

Campbell, G.L., Teitge, D.A. & Classen, H.L. (1991). Genotypic and environmental differences in rye fed to broiler chicks with dietary pentosanase supplementation. *Canadian Journal of Animal Science* 71: 1241-1247.

Campbell, R.C. (1981). Statistics for Biologists. (2nd Ed.). Cambridge University Press, Cambridge.

Candy, D.J. (1980) Biological functions of carbohydrates. pp.138-143. Blackie, London.

Carré, B. (1990). Predicting the dietary energy value of poultry feeds. in Feedstuff Evaluation. Eds: J. Wiseman & D.J.A.Cole. pp.283-300. Butterworths, London

Champ, M., Szylit, O., Raibaud, P. & Aut-Abdelkader, N. (1983). Amylase production by three lactobacillus strains isolated from chicken crop. *Journal of Applied Bacteriology*. **55**: 3, 487-493.

Chesson, A. (1990) Nutritional significance and nutritive value of plant polysaccharides. in Feedstuff Evaluation. Eds: J. Wiseman & D.J.A.Cole. pp.179-196. Butterworths, London

Chesson, A. (1993). Feed enzymes. *Animal Feed Science and Technology*. **45**: 1, 65-79.

Choct, M. & Annison, G. (1990). Anti-nutritive activity of wheat pentosans in broiler diets. *British Poultry Science*. **31**: 811-821.

Choct, M. & Annison, G. (1992). The inhibition of nutrient digestion by wheat pentosans. *British Journal of Nutrition*. **67**: 123-132.

Chojcecki, A.J.S., Gale, M.D. & Bayliss, M.W. (1986). The number and size of starch granules in the wheat endosperm and their association with grain weight. *Annals of Botany* **58**: 819-831.

Classen, H.L., Campbell, G.L., Rossnagel, B.G., Bhatti, R. & Reichert, R.D. (1985) Studies on the use of hulless barley in chick diets: deleterious effects and methods of alleviation. *Canadian Journal of Animal Science*, **65**: 725-733.

Cobb (1995). Cobb 500 Broiler Performance. The Cobb Breeding Co. Chelmsford, Essex.

Cochran, W.G. & Cox, G.M. (1957). Experimental Designs. (2nd Ed.) Chapter 14. John Wiley & Sons, Inc. Canada.

Colonna, P., Leloup, V. & Buleon, A. (1992). Limiting factors of starch hydrolysis. *European Journal of Clinical Nutrition*. **46**: (suppl. 2), S17-S32.

Cowan, D. (1992). Advances in feed enzyme technology. *Agro-Food-Industry-Hi-Tech*. May/June pp. 9-11.

Cowan, D. (1996). Efficiency, performance and stability of enzymes in poultry diets. Personal Communication.

Cutter, E.G. (1978) Plant anatomy Vol.2 (2nd). pp. 264-275. Edward Arnold Ltd, London.

Dale, N. (1994). Matching corn quality and nutritional value. *Feed Mix*. **2**: 1, 26-32.

Dey, P.M. & Dixon, R.A. (1985). Biochemistry of Seed Storage Carbohydrates in green Plant Academic Press, London.

Dixon, R.A. (1985). b-(1-4)-linked glucans from higher plants in Biochemistry of Seed Storage Carbohydrates in green Plant eds. P.M.Dey and R.A.Dixon. Chapter 6. Academic Press, London.

Duffus, C.M. (1993). Starch synthesis and deposition in developing cereal endosperms in Seed Storage Compounds eds. P.R.Shewry & K. Stobart. Chapter 13. Clarendon Press, Oxford.

Duffus, C.M. & Duffus, J.H. (1984). Carbohydrate metabolism in plants. Chapter 2. Longman, Harlow.

Emmans, G.C. (1987). Growth, body composition and feed intake. *World's Poultry Science Journal*. **43**: 3, 208-227.

Englyst, H.N., Anderson, V. & Cummings, J.H. (1983). Starch and non-starch polysaccharides in some cereal foods. *Journal of Science, Food and Agriculture*. **34**: 1434-1440.

Englyst, H.N., Bingham, S.A., Runswick, S.A., Collinson, E. & Cummings, J.H. (1989). Dietary fibre (non-starch polysaccharides) in cereal products. *Journal of Human Nutrition and Dietetics* **2**: 253-271.

Englyst, H.N., Kingman, S.M & Cummings, J.H. (1992). Classification and measurement of nutritionally important starch fractions. *European Journal of Clinical Nutrition*. **46**: (Suppl. 2) S33-S50.

Ernest, T. (1984). Effect of antitrypsin factors of legume seeds on enzyme activity in the digestive tract of chickens. *Roczniki Naukowe Zootechniki, Monografie i Rozprawy*. **22**, 219-253.

Fairley, R.A.C. (1995). An investigation of diet selection as a technique for determining the ideal protein for growing pigs. Ph.D. Thesis. Harper Adams Agricultural College.

Farjo, G.Y., Kloor, I.S. & Ibrahim, I.K. (1986). The effect of diluting broiler's diets with sawdust on performance and digestive tract adaptation to eight weeks of age. *Journal of Agriculture and Water Resources Research, Animal Production*. **5**: 1, 247-263.

Farmer, D.S. (1960). Digestion and the digestive system. in Biology and Comparative Physiology of Birds ed. A.J.Marshall. Academic Press, London.

Farrell, D.J. (1974). General principles and assumptions of calorimetry. pp 1-24 in Energy Requirements of Poultry eds. T.R.Morris & B.M.Freeman. British Poultry Science Ltd., Edinburgh.

Farrell, D.J. (1978). Rapid determination of metabolisable energy of feeds using cockerels. *British Poultry Science*. **19**: 303-308.

Farrell, D.J. (1989). The effects of dietary energy concentration in layers. *Recent Advances in Animal Nutrition in Australia*. pp.8A. Armidale, Australia.

Farrell, D.J., Thomson, E., du Preez, K. & Hayes, J.P. (1989). Advances in the measurement of metabolisable energy in poultry feedstuffs. *Recent advances in Animal Nutrition in Australia*.

Finnfeeds International Ltd. Avizyme: multi-enzyme systems for poultry feed. Technical Services Booklet. Marlborough, Wiltshire.

Finnfeeds International Ltd. Viscosmetric enzyme bio-assay procedure. Technical Services Booklet. Marlborough, Wiltshire.

Freitas, A.R de-, Pieniz, L.C., Michelin-Filho, T. & Rosso, L.A. de-. (1987). Body growth and alimentary tract in broilers. *Pesquisa Agropecuaria Brasileira*. 22: 7, 657-666.

Gallagher, C.H. (1964). Nutritional Factors And Enzymological Disturbances In Animals. pp. 20-24. Crosby Lockwood & Son, London.

Gibson, T.S., Al Qalla, H. & McCleary, B.V. (1992). An improved enzymic method for the measurement of starch damage in wheat flour. *Journal of Cereal Science* 15: 15-27.

Glenn, G.M., Younce, F.L. & Pitts, M.J. (1991). Fundamental physical properties characterising the hardness of wheat endosperm. *Journal of Cereal Science* . **13**: 2, 179-194.

Graham, H. (1994). Foregut digesta viscosity affects broiler performance. *Zootechnica International*. **1**, 54-57.

Graham, H. (1995). A solution to the variable wheat quality problem? *Zootechnica International*. **1**, 52-55.

Graham, H., Åman, P. & Pettersson, D. (1987). Variation in composition of Swedish cereal grains. *Proceedings from 23 Nordic Cereal Congress*. Cereal Science & Technology. pp.87-93. Copenhagen, Denmark.

Gruppen, H. (1996). Triggering the breakdown of nutrients. *Feed Mix*. **4**: 1, 24-28.

Gupta, J.J., Singh, K.S. & Prem-Prakash. (1990). Effect of nature and levels of dietary fibre on the development of gastrointestinal tract in chicken. *Indian Journal of Animal Sciences*. **60**: 10, 1178-1181.

Hagberg, S. (1960). A rapid method for determining alpha-amylase activity. *Cereal Chemistry*. **37**: 218-222.

Hagberg, S. (1961). Note on a simplified rapid method for determining alpha-amylase activity. *Cereal Chemistry*. **38**: 202-203.

Henry, P.R., Ammerman, C.B., Campbell, D.R. & Miles, R.D. (1987). Effect of antibiotics on tissue trace mineral concentration and intestinal tract weight of broiler chicks. *Poultry Science*. **66**: 6, 1014-1018.

HGCA (1993). Wheat quality. *Crops*. November. pp. 9-10

Hill, K.J. (1983). in Physiology and Biochemistry of the Domestic Fowl Vol. 4, Chp. 2. Eds. K.N.Boorman & B.M.Freeman. Academic Press, London.

Hill, F.W. & Anderson, D.L. (1958). Comparison of metabolisable energy and productive energy determinations with growing chicks. *Journal of Nutrition*. **64**: 587-603.

Hong, B.H., Rubenthaler, G.L. & Allan, R.E. (1989). Wheat pentosans II. Estimating kernel harness and pentosans in water extracts by near-infrared reflectance. *Cereal Chemistry* **66**: No. 4, pp. 374-377.

Ishibashi, T., Takenaka, A., Kametaka, M. & Mitsuoka, T. (1983). Dietary effects on digestive enzyme activities in growing, germ-free chicks. *Japanese Poultry Science*. **20**: 3, 170-177.

Jarvis, C.E. & Walker, J.R.L. (1993). Simultaneous, rapid, spectrophotometric determination of total starch, amylose and amylopectin. *Journal of Science, Food & Agriculture*. **63**: 53-57.



Jenner, C.F., Ugalde, T.D. & Aspinall, D. (1991). The physiology of starch and protein deposition in the endosperm of wheat. *Australian Journal of Plant Physiology* **18**: 211-226

Jolly, C.J., Rahman, S., Kortt, A.A. & Higgins, T.J.V. (1993). Characterisation of the wheat Mr 15000 "grain softness protein" and analysis of the relationship between its accumulation in the whole seed and grain softness. *Theoretical Applied Genetics* **86**: 589-597.

Jones, E. (1993). Review. *International Milling Flour & Feed*. **184**: 10, 7.

Jones, G.P.D. & Farrell, D.J. (1992). Early-life food restriction of broiler chickens I. Methods of application, amino acid supplementation and the age at which restrictions should commence. *British Poultry Science*. **33**: 579-587.

Jones, G.P.D., Yang, A. & Farrell, D.J. (1989). Advances in reducing body fat in broilers by nutritional and chemical means. *Recent Advances in Animal Nutrition in Australia*. 332-342. Armidale, Australia.

Kainuma, K. (1988). Structure and chemistry of the starch granule in The Biochemistry of Plants: A Comprehensive Treatise. Vol. 14, Chp. 5. Eds: P.K. Stumpf & E.E. Conn. Academic Press Inc., London.

Kamar, G.A.R., Mourad, H.M., Samy, M.S. & Ahmed, N.A.H. (1987a). Effect of feeding different fibre levels to chickens and ducks on some chemical aspects of the alimentary tract. *Assiut Veterinary Medical Journal*. **16**: 31, 255-266.

Kamar, G.A.R., Mourad, H.M., Samy, M.S. & Ahmed, N.A.H. (1987b). Effect of feeding different fibre levels to chickens and ducks on some physiological aspects of the alimentary tract. 2. Physiological and tissue volumes. *Assiut Veterinary Medical Journal*. **16**: 31, 269-282.

Kessler, J.W. & Thomas, O.P. (1981). The effect of caecectomey and extension of the collection period on the true metabolisable energy values of soybean meal, feather meal, fish meal and blood meal. *Poultry Science*. **60**: 2639-2647.

Kovalenko, N.K., Golovach, T.N. & Kvasnikov, E.I. (1989). Lactate bacteria of the digestive tract of domestic fowl. *Microbiology -New York*. **58**: 1, 116-121, translated from *Mikrobiologiya*, **58**: 1, 137-143.

Larbier, M. & Leclercq, B. (1994). Nutrition and Feeding of Poultry. Nottingham University Press, Loughborough.

Leibetseder, J. (1987). Importance of fineness of grinding and size of pellet for feed intake, digestibility and health in pigs and poultry. *Ubersichten zur Tierernahrung*. **15**: 2, 135-151.

- Leitgeb, S. (1990). Physiological importance of the development of microflora in the digestive tract in broiler chickens. *Sbornik Vysoke Skoly Zemedelske v Praze, Fakulta Agronomicka. Rade-B, Zivocisna Vyroba*. 52, 43-50.
- Lenkova, T. & Sintserova, O. (1985). An effective additive to the diet of poultry. *Ptitsevodstvo*. 4, 24-26.
- Levin, (1976) in Digestion in the Fowl Eds. K.N.Boorman & B.M.Freeman, pp. 63-116. British Poultry Science Ltd., London.
- Lewis, D. (1966). Aspects of poultry nutrition. Chapter 4 in Recent Advances in Animal Nutrition ed. J.T.Abrams. J & A Churchill Ltd, London.
- Lin, H.C., Moller, N.A., Wolinsky, M.M., Kim, B.H., Doty, J.E. & Meyer, J.H. (1992). Sustained slowing effect of lentils on gastric emptying of solids in humans and dogs. *Gastroenterology*. 102: 787-792.
- Longstaff, M.A. & McNab, J.M. (1986). Influence of site and variety on starch, hemicellulose, and cellulose composition of wheats and their digestibilities by adult cockerels. *British Poultry Science*. 27: 3, 435-449.
- Longstaff, M.A., Knox, A. & McNab, J.M. (1988). Digestibility of pentose sugars and uronic acids and their effect on chick weight gain and caecal size. *British Poultry Science*. 29: 379-393.

Lund, D. (1984). Influence of time, temperature, moisture, ingredients and processing conditions on starch gelatinisation. *C.R.C. Critical Reviews in Food Science and Nutrition*. **20**: 4, 249-273.

MAFF (1995). Agriculture in the UK. HMSO, London.

Mahmood, S. & Smithard, R. (1993). A comparison of effects of body weight and feed intake on digestion in broiler cockerels with effect of tannins. *British Journal of Nutrition*. **70**: 3, 701-709.

Manners, D.J. (1985). Starch in Biochemistry of Seed Storage Carbohydrates in green Plant eds. P.M.Dey and R.A.Dixon. Chapter 4. Academic Press London.

March, B.E. & Biely, J. (1973). Chemical, physical and nutritional characteristics of different samples of wheat. *Canadian Journal of Animal Science*. **53**: 569-577.

Martinez, V., Jimenez, M., Gonalons, E. & Vergara, P. (1992). Effects of cholecystokinin and gastrin on gastroduodenal motility and co-ordination in chickens. *Life Sciences*. **52**: 2, 191-198.

Martinez, V., Rodriguez-Membrilla, A., Jimenez, M., Gonalons, E. & Vergara, P. (1993) Immunohistochemical differentiation of gastrin and cholecystokinin in gastrointestinal tract of chickens. *Poultry Science*. **72**: 12, 2328-2336.

Mastika, M. & Cumming, R.B. (1987). Effect of previous experience and environmental variations on the performance and pattern of feed intake of choice-fed and complete fed broilers. In: Recent Advances in Animal Nutrition in Australia . Ed. D.J. Farrell. Armidale, Australia.

Matsiulonis, J., Liczmanski, A. & Wojcik, S. (1992). The effect of the physical form of mixtures on selected gastrointestinal tract features and pancreas proteolytic activity in broilers. *Annales Universitatis Mariae Curie Sklodowska. Sectio EE, Zootechnica*. **10**: 239-244.

May, J.D. & Reece, F.N. (1986). Relationship between photoperiod and feed intake to thyroid hormone concentration. *Poultry Science*. **65**: 4, 801-806.

May, J.D., Branton, S.L., Deaton, J.W. & Simmons, J.D. (1988). Effect of environmental temperature and feeding regimen on quantity of digestive tract contents of broilers. *Poultry Science*. **67**: 1, 64-71.

McCracken, K.J., Urquhart, R. & Bedford, M.R. (1993). Effect of heat treatment and enzyme supplementation of barley-based diets on performance of broiler chicks. *Proceedings of the Nutrition Society*. **52**: 50A.

McDonald, P., Edwards, R.A. & Greenhalgh, J.F.D. (1981). Animal Nutrition. (3rd Edn.). Longman Group Ltd, Harlow.

McLelland, J. (1979). Digestive system. pp. 69-165 in Form and Function in Birds Vol. 1. Eds. A.S.King & J.McLelland. Academic Press, London.

McNab, J. (1976) in Digestion in the fowl Eds. K.N.Boorman & B.M.Freeman. pp. 261-284. British Poultry Science, Edinburgh.

McNab, J.M. (1990) Apparent and true metabolisable energy of poultry diets. in Feedstuff Evaluation Eds J.Wiseman & D.J.A.Cole. pp.41-54. Butterworths, London.

McNab, J. (1991). Factors affecting the nutritive value of wheat for poultry. HGCA report no. 43. HGCA, London.

McNab, J. (1992). Barley  $\beta$ -glucans; an anti-nutritional factor in poultry feeding. *Nutrition Research Reviews*. 5: 45-60.

McNab, J. (1993). Digestibility of starch by poultry. *Feed Mix*. 1: 2, 24-27.

McNab, J. (1995). Factors affecting the energy value of wheat for poultry. *Worlds Poultry Science Journal*. 52: 69-73.

McNab, J.M. & Blair, J.C. (1988). Modified assay for true and apparent metabolisable energy based on tube feeding. *British Poultry Science*. 29: 697-707.

McNab, J.M. & Smithard, R.R. (1992). Barley  $\beta$ -glucan: an antinutritional factor in poultry feeding. *Nutrition Research Reviews*. **5**, 45-60.

McNeil, M., Darvill, A.G., Fry, S.C. & Albersheim, P. (1984). Structure and function of the primary cell walls of plants. *Annual Review of Biochemistry* **53**: 625-663.

Mead, R. & Curnow, R.N. (1983). Statistical Methods in Agriculture and Experimental Biology. Chapman & Hall Ltd., London.

Meluzzi, A., Franchini, A. & Giordani, G. (1986). Lactic acid bacteria and bifidiobacteria in diets for broiler chickens. *Avicoltura*. **55**: 3, 54-56.

Miller, W.S. (1974). The determination of metabolisable energy. in Energy Requirements of Poultry eds. T.R.Morris & B.M.Freeman. pp. 91-112. British Poultry Science Ltd., Edinburgh.

Ministry of Agriculture, Fisheries and Food. (1973). Poultry nutrition. Chapter 1. HMSO, London.

Mollah, Y. (1980), ME of poultry diets in relation to cereal carbohydrates and their interactions. Ph.D. Thesis. University of Sydney.

Mollah, Y., Bryden, W.L., Wallis, I.R., Balnave, D. & Annison, E.F. (1983). Studies on low metabolisable energy wheats for poultry using conventional and rapid assay procedures and effects of processing. *British Poultry Science*, **24**: 81-89

Morell, M.K., Rahman, S., Abrahams, S.L. & Appels, R. (1995). The biochemistry and molecular biology of starch synthesis in cereals. *Australian Journal of Plant Physiology*. **22**: 647-660.

Morrison, W.R. & Gadan, H. (1987). The amylose and lipid contents of starch granules in developing wheat endosperm. *Journal of Cereal Science* **5**: 263-275.

Morrison, W.R. (1992). Analysis of cereal starches in Seed Analysis: Modern methods of plant analysis. Vol. 14. pp. 199-216. Eds. H.F.Linskens & J.F.Jackson. Springer-Verlag, Berlin.

Morrison, W.R. (1993). Cereal starch granule development and composition in Seed Storage Compounds eds. P.R.Shewry & K. Stobart. Chapter 12. Clarendon Press, Oxford.

Morton, J. (1967). Guts: The Form and Function of the Digestive System. pp.10-13. The Institute of Biology's studies in Biology no. 7. Edward Arnold, London.

Mul, A.J. & Perry, F.G. (1994). Role oligosaccharides play in animal nutrition. *Proceedings of the 28th Feed Manufacturers Conference*. pp. 7-10. Nottingham.



Muramatsu, T., Takasu, O., Furuse, M., Tasaki, I. & Okumura, J.I. (1987). Influence of the gut microflora on protein synthesis in tissues and in the whole body of chicks. *Biochemical Journal*. **246**: 2, 475-479.

Muramatsu, T., Takasu, O., Furuse, M. & Okumura, J.I. (1988). Effect of diet type on enhanced intestinal protein synthesis by the gut microflora in the chick. *Journal of Nutrition*. **118**: 9, 1068-1074.

Muramatsu, T., Takemura, J. & Okumura, J. (1993). Effects of supplementing the ammonium bicarbonate on protein synthesis in liver and gastrointestinal tract in germ-free and conventional chicks. *Japanese Poultry Science*. **30**: 4, 263-269.

Newcombe, M. & Summers, J.D. (1984a). Feed intake and gastrointestinal parameters of broiler and leghorn chicks in response to dietary energy concentration. *Nutrition Reports International*. **29**: 5, 1127-1136.

Newcombe, M. & Summers, J.D. (1984b). Comparison of the capacity of the digestive tract of broiler and Leghorn-type chicks. *Nutrition Reports International*. **30**: 2, 297-304.

Newcombe, M. & Summers, J.D. (1984c). Effect of previous diet on feed intake and body weight gain of broiler and leghorn chicks. *Poultry Science*. **63**: 6, 1237-1242.

Nicol, N., Wiseman, J. & Gorton, G. (1993). Factors determining the nutritional value of wheat varieties for poultry. *Carbohydrate polymers*. **21**: 211-215.

Nir, I., Melcion, J.P. & Picard, M. (1990). Effect of particle size of sorghum grains on feed intake and performance in young broilers. *Poultry Science*. **69**: 12, 2177-2164.

Nir, I., Nitsan, Z. & Mahagna, M. (1993). Comparative growth and development of the digestive organs and of some enzymes in broiler and egg type chicks after hatching. *British Poultry Science*. **34**: 3, 523-532.

Nitsan, Z., Ben-Avraham, G., Loret, Z. & Nir, I. (1991). Growth and development of the digestive organs and some enzymes in broiler chicks after hatching. *British Poultry Science*. **32**: 3, 515-523.

Novus (1992). Raw Material Compendium. Novus, Brussels.

Oldham, J.D. & Emmans, G.C. (1990). Animal performance as a criterion for feed evaluation in Feedstuff Evaluation. Eds. J. Wiseman & D.J.A. Cole. pp. 73-90. Butterworths, London.

Parker, M.L. (1985) The relationship between A-type and B-type starch granules in the developing endosperm of wheat. *Journal of Cereal Science*, **3**: 271-278.

Parker, R.E. (1986). Introductory Statistics for Biology; Studies in Biology No. 43. (2nd Ed.). Edward Arnold, London.

Parker, W.J. (1990). Application of intraruminal chromium controlled release capsules to the measurement of herbage intake of sheep at pasture. Ph.D. Thesis. Massey University.

Parsons, C.M., Potter, L.M. & Bliss, B.A. (1982). True metabolisable energy corrected to nitrogen equilibrium. *Poultry Science*. **61**: 2241-2246.

Pawlik, J.R., Fengler, A.I. & Marquardt, R.R. (1990). Improvement of the nutritional value of rye by the partial hydrolysis of the viscous water-soluble pentosans following water-soaking or fungal enzyme treatment. *British Poultry Science*. **31**: 3, 525-538.

Perten, H. (1964). Application of the falling number method for evaluating  $\alpha$ -amylase activity. *Cereal Chemistry*. **41**: 3, 127-140.

Petersen, V.E. (1985). Energy content and the protein:energy ratio of the feed and its influence on growth and feed intake of chickens. *Beretning fra Statens Husdyrbrugsforsog*. No. 582. pp.84.

Petterson, D. (1988). Composition and productive value for broiler chickens of wheat, triticale and rye. Ph.D. Thesis. Agricultural University of Uppsala.

Pettersson, D. & Aman, P. (1989). Enzyme supplementation of a poultry diet containing rye and wheat. *British Journal of Nutrition*. **62**: 139-149.

Piekarzewska, A.B., Rosochacki, S.J. & Sechman, A. (1987). Effect of feed intake on the level of triiodothyronine (T3), reverse triiodothyronine (rT3) and thyroxine (T4) in blood plasma of growing chickens. *Archiv fur Geflugelkunde*. **57**: 6, 210-214.

Pinchasov, Y., Fancher, B.I., Burke, W.H. & Jensen, L.S. (1989). Glycolic acid and tryptophan effects on feed intake and hypothalamic indoleamines in chicks. *Physiology and Behaviour*. **45**: 3, 585-589.

Pinchasov, Y., Nir, I. & Nitsan, Z. (1990). Metabolic and anatomical adaptations of heavy-bodied chicks to intermittent feeding. 2. Pancreatic digestive enzymes. *British Poultry Science*. **31**: 4, 769-777.

Rakaoska, M., Raczynska-Bojanowska, K., Macewicz, J., Kupiek, R. & Dumas, Z. (1989). Non-digestible soluble compounds and grain structure of wheat and rye. *Journal of Cereal Science*. **9**: 159-168.

Reddy, C.V. (1993). Anti-nutritional factors in feedstuffs. *Poultry International*. **32**: 1, 64-68.

Riley, W.W. & Austic, R.E. (1984). Influence of dietary electrolytes on digestive tract pH and acid-base status in chicks. *Poultry Science*. **63**: 11, 2247-2251.

Ring, S.G., Noel, T.R. & Bull, V.J. (1993). The structure of the starch polysaccharides and their organisation in the starch granule in Seed Storage Compounds eds. P.R.Shewry & K. Stobart. Chapter 2. Clarendon Press, Oxford.

Rodeheaver, D.P. & Wyatt, R.D. (1984). Effect of decreased feed intake on serum and pancreatic alpha-amylase of broiler chickens. *Avian Diseases*. **28**: 3, 662-668.

Rogel, A.M., Annison, E.F., Bryden, W.L & Balnave, D. (1987). The digestion of wheat starch in broiler chickens. *Australian Journal of Agricultural Research*. **38**: 639-649.

Rose, S.P. (1997). Principles of Poultry Science. CABI, Wallingford (*in press*).

Rose, S.P., Abbas, G., Kettlewell, P.S. & Watts, R.M. (1992). The nutritional value of different wheat varieties for poultry. Personal Communication.

Rose S.P. & Bedford, M.R. (1995). Relationship between the metabolisable energies of wheat samples and the productive performance of broilers. *British Poultry Science*. **36**: 864-865.

Rose, S.P., Kettlewell, P.S., Reynolds, S.M. & Watts, R.M. (1993). The nutritive value of different wheat varieties for poultry. *Proceedings of the Nutrition Society*. **52**: 2, 206A.

Saastamoinen, M., Plaami, S. & Kumpulainen, J. (1989). Pentosan and b-glucan content of Finnish winter rye varieties as compared with rye of six other countries. *Journal of Cereal Science* **10**: 199-207.

Saini, H.S. & Henry, R.J. (1989). Fractionation and evaluation of triticales pentosans: comparison with wheat and rye. *Cereal Chemistry*. **66**: 1, 11-14.

Salah Uddin, M., Rose, S.P., Hiscock, T.A. & Bonnet, S. (1996). A comparison of the energy availability for chickens of ground and whole grain samples of two wheat varieties. *British Poultry Science*. **37**: 347-357.

Salido, G.M, Pedrosa, F. & Lopez, M.A. (1984). Response to feed intake and the cephalic phase of endocrine pancreatic secretion in the chicken. *Anales de la Facultad de Veterinaria de Leon*. **30**: 259-267.

Salmon, R.E. (1984). True metabolisable energy and dry matter contents of some feedstuffs. *Poultry Science*. **63**: 381-383.

Schutte, J.B., De Jong, J., Van Weerden, E.J. & Van Baak, M.J. (1992). Nutritional value of D-Xylose and L-arabinose for broiler chicks. *British Poultry Science*. **33**: 89-100.

Scott, M.L., Nesheim, M.C. & Young, R.J. (1982). Nutrition of the chicken. (3rd) Chapter 2. M.L.Scott & Associates, New York.

Scott, T.A. (1995). Implications of wheat quality and variability for feeding broilers. *World Poultry*. 11: No. 5.

Selvendran, R.R. & Robertson, J.A. (1990). Chemistry of dietary fibre in Dietary Fibre, Chemical and Biological Aspects Eds. D.A.T Southgate, K. Waldron, I.T. Johnson & G.R Fenwick. pp. 27-43. The Royal Society of Chemistry, Cambridge.

Sengar, S.S. (1987). Feed intake and growth rate pattern in White Leghorn chicks maintained on different planes of nutrition. *Poultry Adviser*. 20: 10, 23-27.

Sheshukova, T.A., Ozols, A-Ya. & Bondarenko, V.I. (1984). Influence of a carbohydrate-rich diet on accumulation of sugars in different sections of the small intestine of fed chickens. *Transportnye i obmennye protsessy v kishechnike zhivotnykh*. 125-135.

Shewry, P.R. & Stobart, K. (1993). Seed Storage Compounds. Clarendon Press, Oxford.

Shires, A., Robblee, A.R., Hardin, R.T. & Clandinin, D.R. (1979). Effect of the previous diet, body weight, and duration of starvation of the assay bird on the true metabolisable energy value of corn. *Poultry Science*. 58: 602-608.

Shires, A., Robblee, A.R., Hardin, R.T. & Clandinin, D.R. (1980). Effect of the age of chickens on the true metabolisable energy values of feed ingredients. *Poultry Science*. **59**: 396-403.

Shollenberger, J.H. & Curtis, J.J. (1949) The chemical composition of various wheats and factors influencing their composition. USDA Technical Bulletin 995, October 1949, pp. 1-33.

Sibbald, I.R. & Morse, P.M. (1982). Effects of the nitrogen correction and of feed intake on true metabolisable energy values. *Poultry Science*. **62**: 138-142.

Sibbald, I.R. & Price, K. (1978). The metabolic and endogenous energy losses in adult roosters. *Poultry Science*. **57**: 556-557.

Sibbald, I.R. & Slinger, S.J. (1963). A biological assay for metabolisable energy in poultry feed ingredients together with findings which demonstrate some of the problems associated with the evaluation of fats. *Poultry Science*. **42**: 313-325.

Sibbald, I.R. (1975). The effect of level of feed intake on metabolisable energy values measured with adult roosters. *Poultry Science*. **54**: 1990-1997.

Sibbald, I.R. (1976). A bioassay for true metabolisable energy in feeding stuffs. *Poultry Science*. **55**: 303-308.



Sibbald, I.R. (1977). The true metabolisable energy values of some feeding stuffs. *Poultry Science*. **56**: 380-382.

Sibbald, I.R. (1978). The effect of the duration of the time interval between assays on true metabolisable energy values measured with adult roosters. *Poultry Science*. **57**: 455-460.

Sibbald, I.R. (1979). Metabolisable energy evaluation of poultry diets in Recent Advances in Animal Nutrition eds. W.Haresign & D.Lewis. pp. 35-49 Butterworths, London.

Sibbald, I.R. (1982). The effect of grinding on the true metabolisable energy value of hull-less barley. *Poultry Science*. **61**: 2509-2511.

Sibbald, I.R. (1985). The Determination of True Metabolisable Energy Value. Agriculture Canada, Ottawa.

Simon, J. & Dubois, M.P. (1983). Subtotal pancreatectomy in the chicken: evidence of a diabetic state and effect on the somatostatin cells of the digestive tract. *Diabete et Metabolisme*. **9**: 1, 75-82.

Sinclair, H.M. & Hollingsworth, D.F. (1971). Hutchison's food and the principles of nutrition. (12<sup>th</sup> Edition). pp.279-303. Edward Arnold Ltd, London.

Sklan, D. (1983). Effect of high vitamin A or tocopherol intake on hepatic lipid metabolism and intestinal absorption and secretion of lipids and bile acids in the chick. *British Journal of Nutrition*. **50**: 2, 409-416.

Smit, H. (1968). Gastric secretion in the lower vertebrates and birds. Chapter 135 in Handbook of Physiology Section 6; Alimentary Canal ed. W.Heidel. American Physiological Society, Washington D.C.

Smulikowska, S. (1992). A simple *in vitro* test for evaluation of the usefulness of industrial enzymes as additives to broiler diet based on rye. *Journal of Animal and Feed Sciences*. **1**: 1, 65-70.

Snow, P. & O'Dea, K. (1984). Factors affecting the rate of hydrolysis of starch in food. *American Journal of Clinical Nutrition*. **34**: 2721-2727.

Sonada, T. (1984). Effects of glucose infused into the duodenum and the abdomen on feed intake in chickens. *Japanese Journal of Zootechnical Science*. **55**: 4, 241-247.

Stark, J.R. & Lynn, A. (1992). Biochemistry of plant polysaccharides. *Biochemical Society Transactions* **20**: 7-13.

Stevens, D.B., Vaidyanathan, L.V. & Baldwin, J.H. (1988). Hagberg falling number and breadmaking quality. HGCA Research Review No.2. HGCA, London.

Stone, B.A. & Clarke, A.E. (1992). Chemistry and Biology of (1-3)- $\beta$ -glucans. Chapter 8. La Trobe University Press, Victoria, Australia.

Sugahara, K., Kubo, T. & Tasaki, I. (1985). Involvement of feed intake in the decreased energy retention in chicks fed an arginine-deficient diet. *Japanese Poultry Science*. **22**: 2, 45-54.

Sulaiman, B.D., Brennan, C.S., Schofield, J.D & Vaughan, J.G. (1993). Some biochemical properties of friabilin and polyclonal antibody production. *Aspects of Applied Biology*. **36**: 61-67.

Sykes, A.H. (1983). in Physiology and Biochemistry of the Domestic Fowl. Vol. 4, Chp. 1. Eds. K.N.Boorman & B.M.Freeman. Academic Press, London.

T.T.Kozlowski (1972). Seed biology Vol.1. pp.106-118. Academic Press, New York & London.

Teeter, R.G. & Smith, O.M. (1985). Feed intake effects upon carcass yield and ration digestibility in broilers force-fed five feed intakes. *Poultry Science*. **64**: 11, 2155-2160.

Teitge, D.A., Campbell, G.L., Classen, H.L. & Thacker, P.A. (1991). Heat pre-treatment as a means of improving the response to dietary pentosanase in chicks fed rye. *Canadian Journal of Animal Science*. **71**: 507-513.

Theander, O., Westerlund, E. & Aman, P. (1993). Structure and components of dietary fibre. *Cereal Foods World*. **38**: 3, 135-141.

Tur, J., Esteban, S., Rayo, J.M., Moreno, M., Miralles, A. & Tur, J.A. (1989). Effect of glucocorticoids on gastrointestinal emptying in young broilers. *British Poultry Science*. **30**: 3, 693-698.

Tur, J.A., Rial, R.V., Esteban, S. & Rayo, J.M. (1986). Ontology of the gastrointestinal motility in young broilers. *Comparative Biochemistry and Physiology*. **83**: 1, 61-65.

Tur, J.A., Rial, R.V. & Pomar, A.M. (1985). Gastrointestinal transit and emptying in fed and fasted chickens. *Comparative Biochemistry and Physiology*. **82**: 2, 329-332.

Tur, J.A., Rial, R.V., Rayo, J.M., Tarraga, S.A. & Dameto, M.C. (1988). Developmental study of digestive motor response to dietary pattern in young broilers. *Comparative Biochemistry and Physiology*. **90**: 2, 241-247.

Tur, J.A., Rial, R.V., Tur, J. & Planas, B. (1987). Effect of thyroidal state on the gastrointestinal transit and emptying of young broilers. *Comparative Biochemistry and Physiology: a Comparative Physiology*. **87**: 3, 665-670.

Van Beek, E. (1991). Kenzyme dry: its effect on feed raw materials. *Feed Compounder*. **11**: 2, 35-37.

Van der Klis, J. & Geerse, K. (1993). The effect of endoxylanase on the nutritive value of different wheat varieties for broilers. *Proceeding of the Society of Chemical Industry, trace components of animal feeds*. London: SCI.

Vincent, J.F.V., Khan, A.A. & Liu, J-H. (1995). The shape of the wheat kernal and its' influence on fracture. *Proceedings of the Royal Society of Chemistry-Food Chemistry Group "Wheat Structure, Biochemistry And Functionality"*. Royal Society of Chemistry, Reading.

Warchalewski, J.R., Madaj, D. & Skupin, J. (1989). The varietal differences in some biological activities of proteins extracted from flours of wheat seeds harvested in 1986. *Die Nahrung*. **33**: 9, 805-821.

Ward, A.T. & Marquardt, R.R. (1987). Antinutritional activity of a water-soluble pentosan-rich fraction from rye grain. *Poultry Science*. **66**: 1665-1674.

Wardroup, P.W. (1988). Dietary nutrient allowances for chickens, turkeys. *Feedstuffs*. **60**: 31, 66-72.

Westerlund, E., Theander, O., Andersson, R. & Aman, P. (1989). Effects of baking on polysaccharides in white bread fractions. *Journal of Cereal Science* **10**: 149-156.

Whistler & Daniel (1979) in Food chemistry Ed. O.R.Fennema. (2nd) pp.91-125. Marcel Dekker, New York.

Williams, C.H., David, D.J. & Iismaa, O. (1962). The determination of chromium oxide in faeces samples by atomic absorption spectrophotometry. *Journal of Agricultural Science*. **59**: 381-385.

Wiseman, J. (1990) Quality requirements of wheat for poultry feed. *Aspects of Applied Biology, Cereal Quality II*. **25**: 41-51.

Wiseman, J. (1993). The nutritional value of wheat in pig and poultry rations. *Agronomist*. **5**, 12-13.

Wiseman, J., Nicol, N. & Norton, G. (1993). Biochemistry and nutritional characterisation of wheat varieties for young broilers. *Aspects of Applied Biology*. **34**: 329-335.

Wiseman, J., Nicol, N. & Norton, G. (1994). Developments in the nutritional value of wheat for non-ruminants. *Proceedings of the 28th Feed Manufacturers Conference*. pp.23-32. Nottingham.

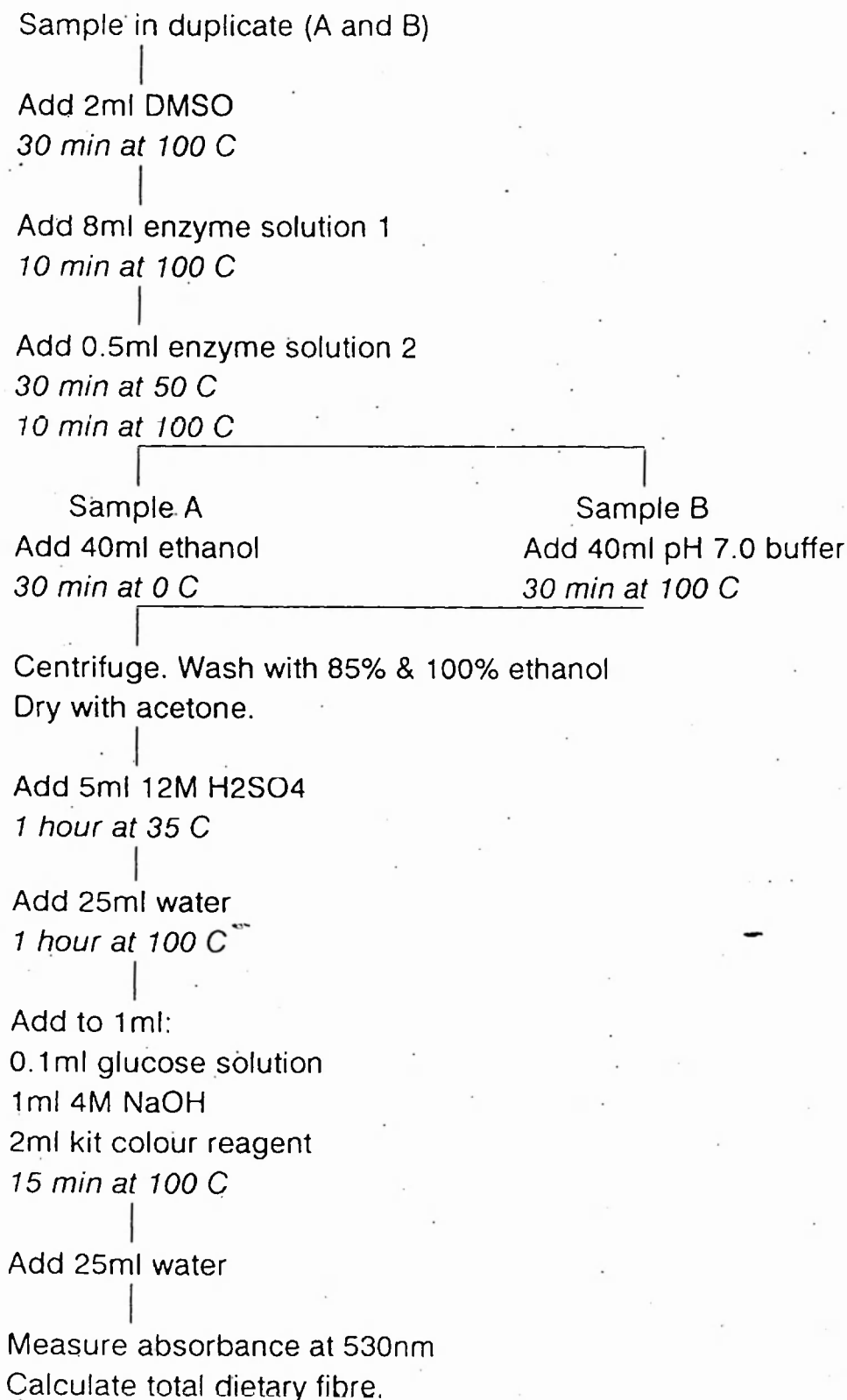
Yamazaki, M. & Aizawa, T. (1986). Studies on the biological assay for the metabolisable energy in poultry feed ingredients. Effect of amount of feed intake and feeding frequency on metabolisable energy. *Japanese Journal of Zoological Science*. **57**: 5, 390-394.

Yo, T. & Tawfik, E.S. (1989). Influence of dietary energy density on feed intake, feed efficiency and weight gain of broilers. *Tropenlandwirt*. **90**: Oct, 1.5-110.

Zaki, A.A., Fatma, A., Elnagmi, A. & Saleh, S.Y. (1993). Effects of selenium and vitamin E on some enzymatic activities of gut contents and sera of chicken. *assiut Veterinary Medical Journal*. **29**: 57, 17-25.

## APPENDIX I

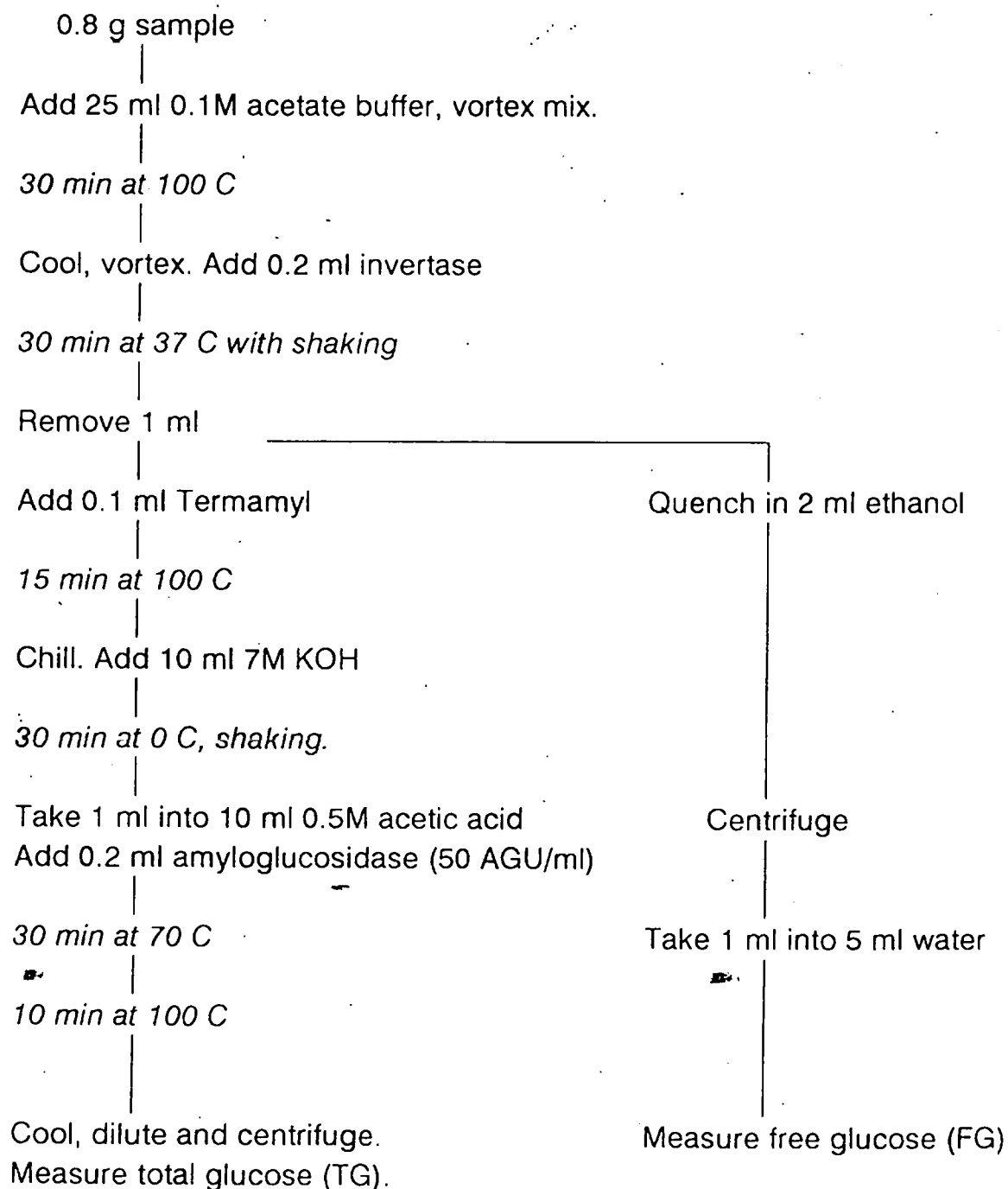
### MEASUREMENT OF TOTAL (A), SOLUBLE AND INSOLUBLE (B) NSP IN WHEAT SAMPLES



*Method devised by Englyst et al, 1992*



## MEASUREMENT OF TOTAL STARCH AND FREE GLUCOSE IN A SAMPLE AS EATEN

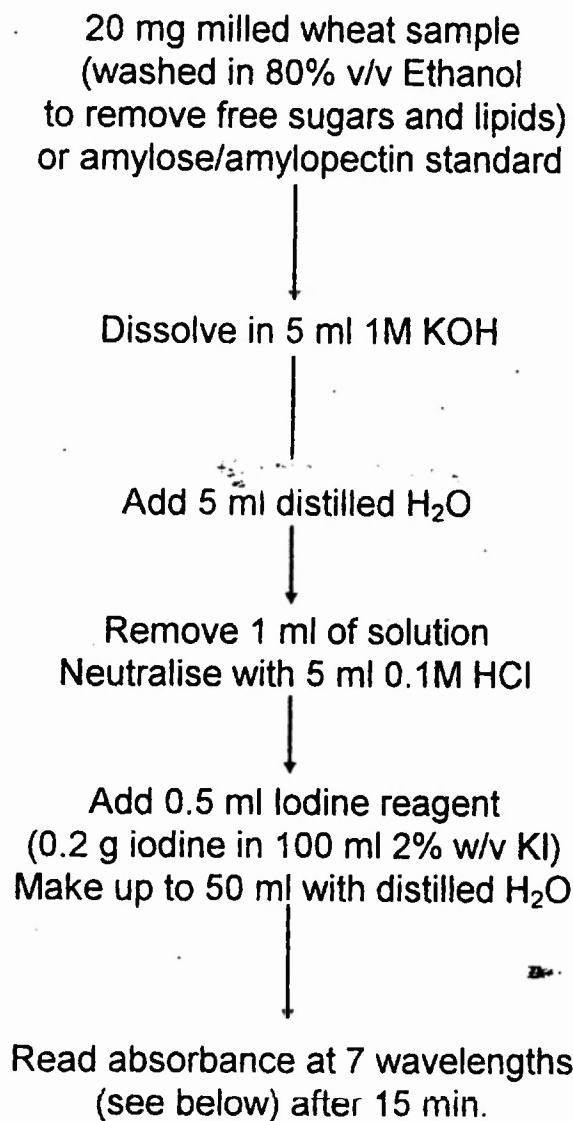


$$\text{TOTAL STARCH} = (\text{TG} - \text{FG}) \times 0.9$$

*Englyst et al, 1992*

## APPENDIX 3

### MEASUREMENT OF AMYLOSE:AMYOPECTIN RATIO IN WHEAT



*Jarvis & Walker, 1993*

Sample Program in Quattro Pro (equivalent to LOTUS 123)

Column A	B	C	D	E	F	G	H	I	J	K	
Row	$\lambda$	$A_{12}$	$A_{ap}$	$A_{am}$	$E_{012}$	$A_{am} \cdot [I_2]$	$A_{ap} \cdot [I_2]$	$E_{0ap}$	$E_{0am}$	$A_{sample} \cdot [I_2]$	$A_{sample}$
1	288	1.379	1.3068	1.0532	B1/0.025			(=G2/5)	(=F2/5)		
2	504	0.025	0.2124	0.3963	B2/0.02	D2-(E2*(D1/E1))	C2-(E2*(C1/E1))	G2/0.04	F2/0.04	K2-(E2*(K1/E1))	
3	548	0.009	0.2405	0.5763	B3/0.02	D3-(E3*(D1/E1))	C3-(E3*(C1/E1))	G3/0.04	F3/0.04	K3-(E3*(K1/E1))	
4	580	0.003	0.2145	0.7582	B4/0.02	D4-(E4*(D1/E1))	C4-(E4*(C1/E1))	G4/0.04	F4/0.04	K4-(E4*(K1/E1))	
5	630	0	0.1486	0.8435	B5/0.02	D5-(E5*(D1/E1))	C5-(E5*(C1/E1))	G5/0.04	F5/0.04	K5-(E5*(K1/E1))	
6	700	0	0.0742	0.7736	B6/0.02	D6-(E6*(D1/E1))	C6-(E6*(C1/E1))	G6/0.04	F6/0.04	K6-(E6*(K1/E1))	
7	720	0	0.0564	0.7137	B7/0.02	D7-(E7*(D1/E1))	C7-(E7*(C1/E1))	G7/0.04	F7/0.04	K7-(E7*(K1/E1))	
#				A/ conc	$A_{am} \cdot (E_{012} \cdot$	$A_{ap} \cdot E_{012} \cdot$		calc	calc	Sample A-(E012	
#					$A_{am}288/E_{012}288)$	$A_{ap}288/E_{012}288)$		$A_{ap}/[ap]A_{am}/[nm]$		*sample A288/ E012 288)	

Final results;

25	mean	@avg(C10..C24)	@avg(D10..D24)
26	std	@std(C10..C24)	@std(D10..D24)

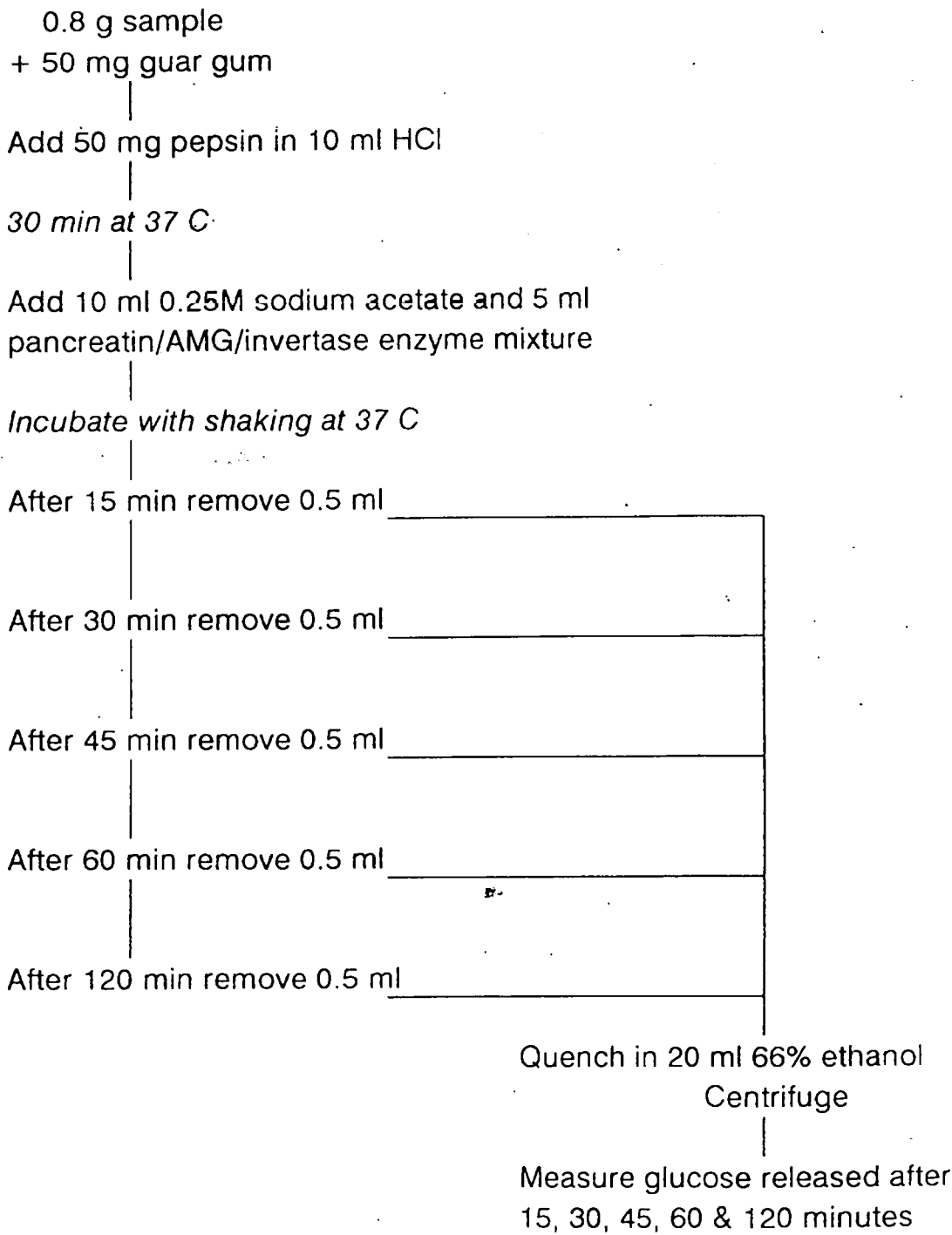
Notes.

1. Columns B, C, and D are the absorbances determined from standard iodine, amylose and amylopectin solutions.
2. \$ represents the conc (mg/ml) of iodine, amylose or amylopectin.
3. Column K is for the actual sample absorbancies.
4. # These lines tell you what the calculation is doing.
5. Starches known to contain lipid must be defatted before analysis.

Reference; J.Sci. Food Agric. 1993, 63, 53-57.

APPENDIX 4

MEASUREMENT OF RATE OF STARCH DIGESTION  
IN WHEAT SAMPLES



*Modified from method devised by Englyst et al, 1992*